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The report is an effort to assemble the best available published procedures for quantitative ecological studies in marine intertidal benthic environments, applicable to evaluating existing or potential pollution effects. Considerable material on how to rigorously devise sampling programs is included. Methods and procedures recommended are synthetic and compiled from a variety of sources.

Guidelines are intended for application in marine intertidal ecological impact assessments, pre- and post-pollution baseline surveys and long term monitoring programs intended for the detection and forecasting of potential and human impacts in the intertidal.

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PROCEDURES FOR QUANTITATIVE ECOLOGICAL ASSESSMENTS IN INTERTIDAL ENVIRONMENTS

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report provides procedural guidelines developed for the special requirements of quantitative ecological investigations in intertidal benthic rocky and sedimentary environments.

A.F. Bartsch
Director, CERL

ABSTRACT

Procedural guidelines developed for the special requirements of quantitative ecological investigations in intertidal benthic rocky and sedimentary environments are given in this report. Community and population characteristics of intertidal macrofaunal and macrophyte assemblages and their relation to possible effects of artificial disturbances are emphasized. The sampling guidelines are applicable to assessments of chronic or episodic impacts, pre- and post-impact baseline studies and long term monitoring programs. Quantitative estimation of natural spatial and temporal variations and distinguishing them from the effects of perturbations are identified as major problems in all intertidal zone pollution investigations.

Methods for developing appropriate and efficient sampling designs through consideration of program objectives and comparisons of relative yields and costs or other constraints are given. Sampling designs stratified by tidal level are recommended and methods for allocating sampling points among strata are explained. Practical aspects of site levelling, surveying and sampling point location are discussed for different shore types as are methods of securing quantitative samples from these biotopes. Photographic methods are recommended for their relative efficiency in non-destructive quadrat sampling on rocky shores.

Specific sampling program development steps are outlined for tide flats, sandy beaches, rocky shores and gravel and cobble shores. A discussion on deriving and analyzing data from sampling programs emphasizes the use of various types of information and data analysis methods in intertidal pollution assessment studies.

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SECTION I

GENERAL INTRODUCTION

"Although much descriptive material on sea shore ecology has been published, there is no general work dealing comprehensively with sampling techniques" N. A. Holme, 1971.

The objective of this study was to assemble the best available published procedures for quantitative ecological investigations in marine intertidal benthic environments, applicable to evaluating existing or potential pollution effects. Early in the study, literature search showed that the statement quoted above from the IBP handbook on methods for the study of marine benthos (Holme & McIntyre, 1971), continues to be correct. There have been no comprehensive original studies devoted to devising or testing methods applicable to intertidal habitats nor reviews critically evaluating published methods for even a major portion of this marine environment. Most of the numerous quantitative intertidal sampling techniques found were devised for limited purposes and published as parts of papers in the very large, diverse and widely scattered primary literature on intertidal ecology. Many methods in current use for studies of the impact of human activities on intertidal environments are found only in unpublished reports of limited circulation. They consequently have not been critically evaluated in the open scientific literature and their validity remains untested.

Because of the nature of the literature and the incompleteness or lack of testing of the methods found there, merely assembling the existing published procedures would not have met the need for a comprehensive guide to intertidal sampling methods. Instead, the methods found were expanded upon from the wider literature on quantitative ecological sampling principles and procedures. The evident lack in many intertidal studies of a sound, objective basis for the design, type and number of samples taken has led us to the inclusion of much material on how to devise rigorous sampling programs, rather than extensive and explicit directions on how to execute plans from the literature, which were originally designed for specific or unique requirements. Adaptation of contemporary methods and standards in quantitative ecological work to designs meeting the special requirements for work in intertidal habitats required a synthesis. The resulting procedures recommended here were compiled from parts of the publications of many workers. It was impossible or unwieldy to supply attributions to all parts of these recommended designs, but the lack of specific citations does not imply a claim of originality by the compilers of this report for the ideas used herein.

Intended uses and users. These guidelines to intertidal sampling are intended for application in marine ecological impact assessments, pre- and post-pollution baseline surveys and long term monitoring programs aimed at detecting and forecasting potential and actual impacts on the intertidal zone. They are intended for use as procedural guides by project managers and scientific personnel who must design and perform such studies but who may have had no prior experience in intertidal work and who lack familiarity with the original literature in intertidal ecology.

Limits of coverage. Procedures given here are primarily for the determination of macrofauna and macrophyte community and population composition and abundance characteristics, relatable to pollution effects. The methods are intended for the reliable estimation of conditions at the time of sampling, with emphasis on distinguishing natural spatial and temporal variation from levels of difference due to perturbations, by quantitative means which can meet rigorous critical challenge. Very large scale, overall survey techniques like that of Ellis (1966) are not considered.

The important ecological properties of rates of primary and secondary production in the intertidal, also susceptible to pollution effects, are covered mostly by reference to literature. Meiofauna, microalgae and bacteria, also of great ecological importance, require field sampling methods different from those given here.

The procedures given are specifically for rocky shores and shores of unconsolidated sediments, both flats and open coast beaches. They may be adaptable to salt marshes, mangrove swamps, coral reefs and other distinctive intertidal environments, but the special problems in these situations require additional methods not specifically covered.

Omitted also are methods for the measurement of physical and some biological features of these shores well covered elsewhere. Methods for sediment analysis, phytobenthos primary production and energy flow measurements in particular are dealt with well in the IBP handbooks on methods for study of the marine benthos (Holme and McIntyre, 1971) and for the measurement of primary production (Vollenweider, 1974). Also excluded are methods for the study of biological interactions, another important aspect of intertidal communities potentially sensitive to pollution effects. For rocky shores, some methods for detecting and measuring biological interactions are given in Connell (1972, 1974), Dayton (1975) and Paine (1974). Predation effects on intertidal soft bottom macrofauna was similarly examined by Woodin (1974) and by Reise (1977).

We have omitted entirely a description of a quasi-quantitative method which has been used for pollution assessments on rocky intertidal areas in Great Britain, because we strongly recommend against its use. This method was intended to overcome the time and effort requirements resulting from intertidal community richness combined with the high degree of spatial heterogenity characteristic of these shores. The quantification of organisms is by scale estimates of numerical density or percent cover. The abundance scales used are given in quantitative terms but their application is by subjective estimates. The results are inherently not comparable in time or space by quantitative statistical methods and operator differences cannot be resolved by testing the resulting data. When such data is converted to histograms in publication, an impression is given of quantitative objectivity which is illusory and subject to serious misinterpretation. This method, its abundance scales and associated wave exposure scale is given in the following: Ballantyne, 1961; Moyse and Nelson-Smith, 1963, 1964; Nelson-Smith, 1977; Crapp, 1971 and Baker, 1976 a and b.

Objectives of pollution related intertidal ecological studies. Field studies of pollution impacts on intertidal habitats will range from assessments of catastrophic episodic events like oil spills, through studies on the degree and geographic extent of lesser, chronic impacts to baseline and monitoring programs in unaffected areas. The goal of the latter category is the early detection of potential future alterations or of estimating potential impacts for regulation of permissable activities.

Common to all of these program types should be the objective of adequate quantitative definition of the state of intertidal communities at the sites of interest so that impact effects are distinguished from natural variations when comparisons are made with previous conditions at the sites or contemporaneous conditions at control sites demonstrably free of impact.

For this quantification, the sampling study design and the methods used must be scientifically rigorous so that the resulting data and conclusions can bear critical scrutiny. Such studies will be under scientific review by cognizant governmental agencies as well as many other bodies with scientific capability, often under conditions of contested hearings or judicial review. Guidelines for the design of sampling programs and the selection of quantitative methods suitable for meeting the objective defined above are provided in this compendium. These methods will be suitable for: a. assessing the ecological effects of acute, single episodes of disturbance or pollution by comparison to control sites, b. the establishment of quantitative baseline information on critical or sensitive ecological areas to be used in detecting the onset of pollution impacts, c. monitoring studies designed to detect both progressive changes from chronic pollution and recovery processes following abatement of ecological stress.

Programs in the above areas should employ the following criteria during planning. Study designs and methods must be capable of quantitatively differentiating between impact sites and non-polluted areas, with adequate definition of natural spatial variations or heterogenity and also natural temporal fluctuations. They must be capable of discriminating natural long term and seasonal changes from progressive alterations due to chronic or increasing impacts. Comparative temporal studies should result in a data base on rates and sequences of degradation and recovery processes suitable for predictive use.

Special features of intertidal sampling

Accessibility. The planner of intertidal sampling programs is confronted with severe time constraints on access to this region, imposed by the diurnal and seasonal variations in the time and level of low tide. Both the frequency and duration of tidal exposure changes down the tidal gradient. On the Pacific coast of North America for example, only on about one-third of the days in a year does the tide level at low tide approximate the mean lower low water level (0.0, MLLW). Accessibility to most of the vertical tide range on a single low tide exposure is consequently limited to relatively few days and sampling at different levels on different days becomes a practical necessity. The lowest low tides not only produce exposure of the lower levels but also longer available working times at higher levels. Since the duration of exposure of low levels is short as well as infrequent compared to upper levels, priority must be given to lower level work. Inevitably other intervening factors interfere with sampling schedules for the lower levels, such as high storm surf which may make lower levels inaccessible at times set for winter seasonal sampling.

Biotic stratification or zonation. The major spatial feature of the distribution of intertidal species is their discreet vertical limits within the tidal range. This results in an overall stratification or zonation of the community with respect to tidal height, because of the approximate coincidence of many groups of these vertical limits. The ultimate cause of zonation limits and the large number of factors modifying them on rocky shores is the subject of a large literature not reviewed here, which identifies both biotic and physical causes and interactions between the two. The many variations in intertidal zonation are one of the major sources of natural spatial variation on the large scale between sites, which must be adequately accounted for in pollution assessment, especially in the selection of control sites. While most attention has been devoted to zonation on rocky shores, discrete vertical species limits are also characteristic of beaches and other sedimentary shores, but are not apparent from surface inspection alone.

Since vertical tide range limits of species and changes in their density with tidal height are universal features of all shore types, it is recommended that these known discontinuities and their direction be reflected in sampling designs, and that the tidal heights of all sample units be carefully identified in any type of sampling design used.

In the face of known vertical biological stratification, any sampling plan which utilizes horizontal distances alone for locating sampling points will confound vertical and horizontal causes of differences between points unless a vast number of sample units are used. Moreover, such designs severely limit the usefulness of comparisons between sites or times.

Since on all types of shores the slope is not constant across the shore, transects across shores, stratified by vertical height, are the most generally applicable bases for sampling designs for the intertidal. As a rule, one foot (0.3 m) vertical limits to strata offer ample resolution of population and community changes with vertical height. Allocation of sample units among strata of unequal widths, resulting from slope differences, is dealt with in later sections. A stratified sampling design, with strata parallel to the shoreline and defined by tidal height contours or by obvious vertical limits of species associations, also defines hypotheses about vertical distributions and abundances within and between sites which statistical testing of the sample data may refute or support. In turn, any analysis which, in order to make comparisons with other sites or sampling periods, combines all data for all levels within intertidal transects, will probably not reveal existing differences between sites, because the within-transect variance produced by combining data from different levels will be enormous. An appropriate analysis will compare strata of similar tidal height from different sites.

An example illustrating the points above for rocky shores is the study of Batzli (1969) who compared the relative biomass of the species populations and species composition in two transects of tide pools stratified by tidal height. While tide pools tend to minimize differences between heights because they hold water at low tide; Batzli found that species number, biomass and its distribution among species, and equitability as an index of the distribution of relative abundances of species were all related to the tidal height of the pools. He further noted that this distribution along the natural environmental gradient of stress resembled that resulting from pollution stress gradients. A similar study by Glynn (1965), showed similar community changes with tidal height over a vertical distance of approximately one meter.

II. SAMPLING PROGRAM DESIGN CONSIDERATIONS

INTRODUCTION

The role of field sampling in ecological studies is to produce unbiased, reasonably precise estimates of population parameters without having to census entire populations. An appropriate sampling design permits detection, within specified limits of accuracy and precision, of changes in population or community parameters. The selection of a sampling design is complex. Methods have been developed to aid the selection of the most efficient design through comparison of relative costs and yields. A discussion of the factors affecting selection of a sampling design may be approached by considering the nature of the environment to be studied.

The intertidal substrate ranges from mud to sand, pebble, cobble, or consolidated rock, and grades or mixtures between these. Each substrate has unique properties which influence the sampling process. Common to all substrates is heterogeneity and the gradient of intertidal elevation. Generally, the shoreline is broken into sections by natural or man made boundaries such as headlands or jetties. A problem often presented in ecological investigations is to characterize the assemblage of organisms within a bounded section of the shore. Differences between areas or changes in one location through time cannot be interpreted unless they can be distinguished from the spatial and temporal variations which are normally present at the locations. An important part of field sampling, therefore, is quantification of spatial and temporal heterogeneity.

Changes in environmental factors or in the density of organisms may occur over a range of distances or time intervals, which may be loosely referred to as the spatial or temporal "scale" of variation. At a given location, several scales of variability may be present. The degree of spatial and temporal variation present at a range of scales must be identified to accurately assess differences between samples.

The number of scales on which sampling must be applied requires clearly defined terminology. The following terms will be used.

Sampling location, site or area: a section of the shoreline delimited by natural or man made boundaries so as to be easily distinguished.

Transect: any representative part of the sampling location, usually extending from the high water line to the low water line, or the full width of the intertidal zone.

Stratum: divisions of the transect usually by elevation, which are more variable between than within themselves.

Common shore terminology is not standardized and can be confusing because three, not two, dimensions are the references to points on a shore. The terms used here are as follows.

Width or horizontal depth of the shore: the distance on a shore from the uppermost high tide line, directly across the beach slope toward the lowermost tide or water line.

Tidal height, vertical level or tidal level: the elevation of a point in the intertidal zone of the shore relative to sea level.

Length of the shore, or the alongshore directions: the horizontal distance on a shore parallel to the uppermost high tide line and lowermost low tide line. The topographic contours representing vertical tidal elevations run along the length of a shore, demarking slope changes across its width.

Each transect is one representative part of the sampling location, regardless of the number of cores or quadrats taken within the transect. If the sampling location can be assumed to be nearly uniform on a scale of meters (the usual scale of transects), then one randomly positioned transect would give a reasonable representation of the location. However, if the location is more heterogeneous, then transects in different parts of the sampling location may produce different conclusions. In most cases lack of foreknowledge forces two or more transects to be selected in any given location, unless the location is very limited in area. The determination of the number of transects to be sampled will be discussed in a later section, following discussion of the basics of the selection of sample unit and sample sizes. Positioning of transects is usually arbitrary, or randomized along a line much longer than the transect is to be wide.

A transect can vary from a line of potential places to put a sample unit, to a wide belt within which sample units are positioned according to various designs. The width of the transect is a function of the available homogenous area. When possible, transects are usually from twenty to thirty meters in width. In the rocky intertidal the widest space available, free of inconvenient changes in tidal height, outcrops or deep channels may be only one or two meters wide, and the transect is correspondingly narrower.

The transect width should be responsive to the suspected important scale of variation at the given location. When variations in population density or environmental factors occur on a scale of meters, then the transect should be meters wide. When the only major variation is on a scale of less than one meter, the transect may be reduced to a line of quadrat positions. However, if no prior information is available on possible scales of variability, transects should not be reduced to lines of sample units. Specific recommendations will be made for the various substrates in later sections.

The following terms will be used repeatedly in the remainder of the discussions.

Sample unit = a single quadrat or core, which defines the area or volume within which the measurements on organisms are to be taken.

Sample unit size = the area or volume of the sample unit.

Sample size = the number of sample units in one sample.

Sample = a representative selection of the total population of organisms, which is used to estimate population and community parameters within the area in which the sample was taken. A transect may be sampled using one set of sample units (one sample). If this sample is allocated to strata within the transect, each stratum has a separate sample; the collection of samples within strata still represent only one sample of the transect.

Two major types of sampling are commonly applied to the intertidal. These are systematic and random sampling. The former spaces sample units (quadrats) evenly in the area of the transect, or within each stratum of the transect. The latter randomly places the sample units over the area of the transect, or in each stratum of the transect. The fundamental procedure in either case is unchanged for line or belt transects. The principal advantages and disadvantages of the two methods as summarized by Cochran (1977) are:

1. Systematic sampling is simpler in execution. A single sample unit position must be randomly selected. All other sample units are

automatically positioned according to the size of the area and the desired number of sample units. Random sampling, in contrast, requires that each sample unit have a random position assigned to it. Both the selection of positions and their location in the field are usually more time consuming than for systematic sampling.

2. In some cases, especially with a small sample size, the greater spread of a systematic sample can result in a better estimate of the mean than that from a random sample of similar size.

3. Periodicity or linear trend in the variable under study, such as the abundance of species, will often result in over or under estimation of the mean of the variable, depending on the degree to which the systematic sample is spaced coincidentally with the period of the study variable or on its position relative to the central value of a trend in the variable. Further discussion of this problem is found in Kelley (1976).

4. There is no reliable method for the estimation of the variance of the mean of a single systematic sample. At least two systematic samples must be taken within each transect, or within each stratum, for the estimation of the variance of the mean. For k independent systematic samples, the estimate of the variance would have k-1 degrees of freedom. A single random sample, in contrast, has n-1 degrees of freedom for the estimate of variance, where n is the number of sample units. The estimate of the variance tends to be erratic when based on a small number of degrees of freedom. Systematic sampling can be prohibitive when a large enough number of samples are taken to yield an adequate estimate of the variance. In general, random sampling is superior to systematic sampling for the type of study required in the assessment of pollution effects, in which the variation of the mean number of individuals of each species is important. Further discussion of this topic may be found in Cochran (1977).

One of the most difficult parts of the development of a sampling program is the selection of the sample size, which is closely linked to the selection of the size of the sample unit. The size of the quadrat used must reflect the nature of the species present at the sample location. Important large or rare organisms may be missed by a small quadrat. Large quadrats, however, are generally more expensive in time and effort involved in the sampling and sorting process. For each quadrat size which is adequate to sample the species present at a given site, the cost and efficiency are calculated. It may be that a smaller quadrat is less costly than the number of larger quadrats required to produce the same precision of estimation, but that the larger quadrat returns a greater number of species. The criteria for selection of an appropriate sample unit size and sample size are discussed in detail in later sections.

For the application of the formulae which are used to determine the relative efficiency of sample units and sample sizes, some prior information on the sample location is necessary. This information is normally obtained either from previous work in the same or similar locations, or from a preliminary sample. The preliminary sample is necessarily inefficient, because it is not optimal for the given conditions. The key part of the information required to produce an efficient sampling program is the variability of the area. The initial survey must gain a reasonably precise estimate of the variability of the area at the time of the survey, and thus should be larger than the final program rather than smaller. Since the initial survey is not repeated, the loss of time or other expense is more than compensated for by the efficiency of the final program.

In following sections the terms mean and variance will be frequently used. In most cases, intertidal studies will involve a number of species, each with an associated mean density and the variance of that mean. In methods such as the determination of sample size, or the allocation of sample units to strata, a single mean and variance is used. The calculations could be repeated separately for each of the species for which the mean and variance are known. In practice, the calculations are made for each of the species which seem interesting, and various averaging methods are used if the results for different species are widely discrepant. An alternative is to accept a loss in the efficiency of sampling some species in exchange for a greater ability to sample others. Since the methods are approximate, fairly crude means of averaging produce sampling programs which are quite able to detect changes at the desired levels of accuracy and precision. In the following sections the problem of dealing with several species, and therefore multiple independent variables, will be elaborated as necessary.

STRATIFICATION

Stratification is the process of division of an area such as a transect into sub-areas which are presumed to be more variable between themselves than areas within each of the sub-areas. Strata are sampled independently of each other, and the information can be used to compare strata or to form a better estimate of the mean density and variance of species within the entire transect. Excellent examples of the application of stratification procedures in marine benthic work are the studies of Brinkhuis (1976) and Saila et al. (1975), which may be consulted as models.

In the intertidal, the most common method is to stratify by either uniform intervals of elevation, or by obvious discontinuities in the community of organisms. When the transect is stratified by either method , the estimate of the variance of the mean density of any species over the entire transect is superior to that which would result from an unstratified sample. If the communities within the strata are greatly different, the strata may be treated as separate populations and their species compositions and densities considered separately.

Within any given transect, the sample size n will be defined by the sampling plan. The n units must be allocated to strata. The simplest procedure for allocation is to allocate sample units in proportion to the area of the stratum. The area of the stratum can be represented as the total number of possible contiguous positions for sample units; that is, the area is some multiple of the area of the sample unit to be used. If the total stratum area is N_h, then the number of sample units allocated to the stratum is given by

$$n_h = \frac{n\,N_h}{N} = nW_h, \; W_h = \frac{N_h}{N} = \text{stratum weight}$$

where N is the area of the entire transect expressed as a multiple of the sample unit size. Since the relative areas of strata are compared and used to weight their allocations, any means of expressing their areas could be used (eg- m^2). The purpose of using the sample unit size as the unit of measurement will be discussed below.

This allocation method gives an unbiased estimate of the mean density of any species for the entire transect, when the means within strata are pooled. The pooled estimate of the transect mean density for any given species is the weighted average of the stratum means,

$$\bar{y}_{tran} = \frac{N_1\bar{y}_1 + N_2\bar{y}_2 + \cdots + N_h\bar{y}_h}{N}$$

or the equivalent expression,

$$\bar{y}_{tran} = W_1\bar{y}_1 + W_2\bar{y}_2 + \cdot\cdot\cdot + W_h\bar{y}_h$$

Since the sample units have been allocated proportionally, the stratum weight W_h can be expressed in terms of the stratum sample sizes (the allocations to strata),

$$\bar{y}_{tran} = \frac{n_1\bar{y}_1 + n_2\bar{y}_2 + \ldots + n_h\bar{y}_h}{n}$$

This is true simply because $W_h = N_h/N = n_h/n$. The estimate of the variance of the mean for the entire transect is given by

$$V(\bar{y}_{tran}) = \frac{1-f}{n}(W_1S_1^2 + W_2S_2^2 + \ldots + W_hS_h^2)$$

Here the finite sampling correction $\frac{1-f}{n}$ is included. The sampling fraction f is given by n/N. If the ratio of the total area of the sample units in the sample to the total area of the transect is less than 0.10, then the sample can be assumed to be drawn from an infinite population, and the taking of one sample unit does not measurably affect the probability of taking another sample unit at another position. If the ratio exceeds 0.10, the population is effectively finite. The finite population correction adjusts for the bias introduced by sampling without replacement of units in a finite population. If N is expressed in terms of multiples of the sample unit size, then the sampling fraction is actually the number of sample units in the sample divided by the number of units which could have been contiguously placed in the transect.

The formula for the variance given above uses the true variances of the strata means. In practice, the sample variance estimates the true variance and the formula is

$$s^2(\bar{y}_{tran}) = W_1 s_1^2 + W_2 s_2^2 + \ldots + W_h s_h^2$$

assuming that the finite population correction is not needed.

The method of proportional allocation produces a suitable unbiased estimate of the transect mean and variance for each species, but does not maximize the efficiency with which the mean and variance in each stratum are estimated. Cochran (1977) describes methods for allocation which optimize the estimation of stratum means and variances, with comparison of strata as the primary goal.

A second problem is that proportional allocation does not take into account differences in the variability of a species in different strata. An optimal allocation scheme would assign more sample units to strata where the variance was higher, even if the areas of strata were equal. Estimates of the variance within strata are rarely available from preliminary sampling, so this more refined method would seldom be applied in the intertidal. The Neymann allocation method, one of those which account for differences in variance, is detailed by Cochran (1977). Cochran also discusses the inclusion of differences in the cost of sampling between strata, but this would seldom be important in field studies of the intertidal. In most cases the sampling method will be the same in all strata, and the cost per sample unit is constant for all strata.

A potentially serious problem in stratification is the inaccurate estimation of stratum area. If strata are greatly different in area from the estimated size, then stratification can actually decrease the effectiveness of the sampling program. In the intertidal, such errors could arise by assuming that strata are equal in area because they are equal in the interval of tidal height. Changes in the slope of a shore result in different areas for equal intervals of elevation. The actual areas of strata should be used rather than assuming the strata are of equal area. Final adjustments to allocations may be necessary in the field, following surveying and actual measurement of stratum areas.

A separate allocation design could be computed for each species. If there is no major discrepency between the allocation schemes produced from different species data, a simple arithmetic average of the allocations for each stratum should be sufficient. If some species produce allocations which differ greatly from others, a decision must be made concerning the importance of those species in the sampling program. If they are important, they can be given greater weight in the averaging of the allocations to a given stratum. If not, they can be given reduced weight in the averaging. More complex methods to produce an optimal allocation are possible and some of these are described by Cochran. In practice, the allocation of sample units to strata is sufficiently crude that moderately large discrepencies between the allocations from different species data can simply be averaged.

SYSTEMATIC SAMPLING

For simplicity, assume that the transect has been selected and is to be sampled without division into strata. The transect area may be expressed as N units of the same area as the chosen sample unit. The transect may range from a line of potential positions for the sample units to a larger area within which sample units are positioned. In systematic sampling, the units which are sampled are spaced evenly over the area. The spacing is simply N/n, where n is the number of sample units in the sample. If k = N/n, every kth unit would be sampled to give a total of n units in the sample. In practice, k is often not an integer, so the nearest integer value to N/n is used.

The process of selection for the sample of n units is:

1. From the area of the transect and the area of the sample unit, the value of $N = \frac{\text{total area}}{\text{sample unit area}}$ is determined. Given the value of n required for the program contemplated (see section on sample size selection), the value of k is determined to the nearest integer. In a simple example, suppose that the transect is a line of positions .5 m wide (sample units are .25 m^2) and 100 m long. There are 200 possible contiguous positions in which to place a .5 x .5 m sample unit. If the sample size is to be 10 units, the value of k =200/10 = 20. Every 20th position should be used, or positions 20 x o.5 m = 10 m apart.

2. If the transect is a line of possible positions, the first unit to be sampled is selected from the N possible positions at random. Every kth unit in both directions from the first unit position is also sampled. In the example above, every 20th position from the first in both directions is sampled; or equivalently, sample units are positioned at 10 m intervals from the first unit. The purpose of starting at some random position rather than at the first position in the line is to ensure that the sample is unbiased. If k is not an integer, the last few units might not be sampled if sampling is begun at the first unit. A random start allows any sample unit position to be sampled.

   Note that if the distance separating chosen sample unit positions is used rather than counting off every position, small units can be readily located in a long line. Compare the difficulty of locating 10 positions in a line of 1000 possible positions, to simply measuring off distances and sampling at equal intervals.

3. If the transect is an area rather than a narrow line of possible sample unit positions, the transect may be divided into adjecent rows of potential sampling unit positions extending the width of the transect. The unit positions can be numbered from one to N along adjacent rows. A random position i is chosen, and positions i+k, 1+2k, i+3k, and

so on, and also positions 1-k, 1-2k, 1-3k, ..., would be sampled. A disadvantage to this method is that in the direction parallel to the waterline, the units are sampled at fairly long intervals, whereas in the perpendicular direction the rows are adjacent. The pattern of sample units could be so extreme as a set of columns of sampled positions:

x x x x x
x x x x x
x x x x x

Obviously, the scale of variation measured in the two directions would be different. For most purposes, it is better to have the rows of sampling positions separated by the same distance as separates the units horizontally, forming a grid of squares:

x x x x x

x x x x x

x x x x x

The mean of a systematic sample is calculated in the usual manner. There is no unbiased method for the estimation of the variance of the mean of a single systematic sample. Cochran (1977) gives approximations for the variance of a single sample when the parent distribution follows certain forms, but these are not likely to be encountered in ecological studies. In general, at least two systematic samples are required within each transect in order to estimate the variance. The variance of a set of systematic samples is calculated according to the usual definition of the variance, where the means of entire samples become the observations $\bar{y}i$ on the variable, and the mean of those sample means becomes the variable mean $\bar{Y}$.

$$V(\bar{y}_{sy}) = \frac{1}{K}\sum_{i=1}^{k}(\bar{y}_i - \bar{Y})^2 \text{ for } k \text{ samples.}$$

The estimate of variance will have k-1 degrees of freedom; since k is usually small, the estimate will be somewhat more erratic than the estimate of variance derived from a single random sample with n-1 degrees of freedom.

If the transect is stratified, then the same procedure is followed within each of the strata. At least two systematic samples would be taken within each stratum in order to estimate the variance in the strata, from which the transect variance is calculated. Each sample should ideally have a sample size equal to the number of units allocated to that stratum, but this would result in doubling of the total number of sample units per transect if the minimum of two samples per stratum were taken. The alternatives are to allow each stratum sample to use half the allocation for that stratum, or to halve the number of strata and permit each sample to have about the full allocation of one stratum. The first alternative results in a loss of precision due to inadequate sample size, and the second a loss of precision due to inappropriate stratification. If strata have been selected on an arbitrary height interval basis, then halving of the number of strata is probably less detrimental to the quality of the sampling program than halving the sample size within strata. If the strata are based on very obvious discontinuities, the best solution is to reduce the total number of transects in the program in order to maintain the quality of the sampling within the remaining transects.

RANDOM SAMPLING

The simplest case is when random sampling is used in an unstratified line transect. The transect has N possible positions in which a sample unit could be placed. A sample of n of the total possible N positions is to be selected. In random sampling, every one of the n units is positioned randomly and independently of the others, except that no position is sampled twice. In a line transect, the possible positions are numbered from 1 to N and n of these numbers are randomly chosen and sampled.

When the sample unit is small, and the transect area large, it is difficult to identify every possible position by number. The usual method is to use a subset of the total possible positions by imposing a grid over the area and selecting a random number of points on the grid. For example, suppose that a small core of 5 cm diameter is to be used, and the transect is 30 m wide and 100 m long. There are about 1.5×10^6 possible positions. Instead of using this large number, a large subset is defined. The transect is divided into 0.5 m or 1 m intervals in both directions, forming a grid with either 12,000 positions or 3,000 positions. For easier location of sample unit positions, two random numbers are used to define each position to be sampled. For 1 m spacing of the grid, a random number, x, between 1 and 100 is chosen for the first coordinate, and a second random number, y, between 1 and 30 defines the second coordinate and sample unit position. One simply samples x units from the top of the transect, and y units from the starting side of the transect. To speed the sampling process, all random positions are selected in advance, and the positions along each row of the grid which are to be sampled are listed. Sampling then consists of measuring to the row, and sampling at predetermined distances along the row.

It is essential that the interval of the grid is such that a large number of positions are available, and the sample size n is never more than 10% of the possible number of positions.

When the transect is stratified, the same process is used within each of the strata. Random sampling does not require more than one sample per stratum, so the sample size within each stratum is the allocation to the stratum.

SAMPLE UNIT SIZE

A sample unit is a known area or volume of the sampling location, enclosed by any device or bounded by any other means. A core is a common sampling unit, but imaginary lines around a space 25 m on a side equally define a sampling unit. In a few cases, only parts of the sampling unit area are sampled; these are subsamples of the basic sampling unit, because their position in the transect or in the stratum is determined by the position of the sample unit.

Two major criteria are applied to the selection of an appropriate sample unit size. The simpler is the maximization of the number of species retrieved by the sample. As the sample unit size increases, the sample comprises a greater part of the total area, and the probability of collecting all of the species present in the area increases. The same qualitative effect occurs when the sample size, or number of sample units, increases. Typically, as the sample unit size increases, there is an initially rapid increase in the number of species recovered in samples of the same size, followed by a levelling off at some specific sample unit size. Further increase in the sample unit size above this size yields relatively less of an increase in the number of species recovered, although for any practical range of sample unit sizes, the number of species recovered will continue to increase slowly with the sample unit size. The optimal sample unit will be some unit near to or larger than the size at which the levelling off occurs, by the criterion of maximization of species recovered.

The second criterion is the minimization of the variance of the mean. Most organisms are found to be patchily distributed in the intertidal. It is well known that as the sample unit size changes, the variation in the numbers of a given species between sample units also changes. This property has been used to estimate the size of patches for a given species. As the quadrat size approaches the scale on which patchiness occurs for a given species, the variance of that species will increase. At quadrat sizes greater than the patch scale, the variance again decreases. At some point, the scales of quadrat and patches are so different that the sample may appear to have been taken in a random distribution of that species. At this quadrat size, the variance will be nearly equal to the mean (a characteristic of random distributions), and the estimate of variance will be minimized. Effectively, use of an appropriate sample unit size can decrease the measured variation by eliminating the component of variation which results from patchiness. Since the comparison of locations and detection of differences is largely dependent on the variance of the means of the locations, unnecessary addition to the variance are disadvantageous. Of course, for two such locations to be compared it follows that the quadrat sizes used in both locations must be equal.

Quadrats of different size vary in the cost of taking the sample and processing it. In addition to maximization of the number of species and minimization of the variance, most programs must consider the economics of sampling, and choose a sampling design which is most efficient monetarily within the bounds of the scientific requirements. Cochran (1977) gives a method for the selection of the optimal sample unit size which includes both the cost of each sample unit in use, and the variance of the sample when that sample unit size is used. The "relative net precision" of a unit of a given size is given by $M_u^2/C_u^2S_u^2$, where M_u is the relative size as compared to the smallest unit, C_u is the relative cost as compared to the cheapest unit, and S_u^2 is the variance associated with the unit of size u. The best unit is the one with the highest relative net precision. Of course, each species has a separate variance, so that a different set of relative net precision values would be calculated for each species. If different unit sizes are indicated when the data for several species are used, then some compromise must be made. The simplest method is to sum the relative net precision values for each quadrat size over all species of interest, and choose the unit size with the highest sum. If some species are possibly more important than others, weighting of their relative precision values would be appropriate.

The above method does not take into account the number of species returned by each size of quadrat. The selection of the most efficient sample unit size should be restricted to the range of sizes which satisfy the condition that they recover a large part of the species present in the sampling location. Smaller quadrats may prove to be more economical, but the usefulness of the sample will be limited if the community is not adequately represented.

As was the case for allocation of sample units to strata, preliminary information is required to evaluate the best sample unit size. The initial survey should include sampling with a range of quadrat sizes, each quadrat size used to take an independent random sample in an appropriate area. All samples should be of the same size (have the same number of units). When the approximate range of sizes possible have been determined from the plot of species richness against sample unit size, the efficiency formula above, or an equivalent, should be applied to determine which sample unit size has the best yield in terms of low cost and minimal variance. The range of sizes to be tried in the preliminary survey can be determined by examination of sample unit sizes used under similar conditions. The relative cost of each unit will be determined in application during the initial survey.

In some cases, a few species will be so different in size or abundance that a completely different sample unit will be required. Large starfish, for example, might be sampled by units as large as 100 m^2. The remainder of the species in the area would be sampled using some much smaller quadrat size. Species such as starfish are potentially important to the community and should not be ignored because they are rare. In general, very large fauna often have an important influence on the community which may not be reflected in the total numbers or biomass of these large species.

SAMPLE SIZE

The sample size is the number of sample units in the sample taken within a transect. The total sample size may be allocated among strata. Each stratum sample then has its own sample size, which is the allocation assigned to it.

In determining the total number of units to be taken, however, the sample size of the entire transect is considered first.

The selection of a non-arbitrary sample size has several purposes. The precision of estimation of the mean density of any species increases (the variance decreases) as the number of sample units increases. At the same time, the accuracy of the estimate of the true population mean increases. The total cost of the sample also increases, so that it is desirable to select a sample size which will yield an estimate of the true mean which has the desired levels of accuracy and precision, but which is no larger than absolutely necessary. The required sample size can be calculated for each transect or location, and the total cost for each sample can be estimated. Under a given budget, the total number of samples which can be taken at a location, or the number of locations which can be examined given a specified number of transects per location, can be determined. Unless the budget is unlimited, this sort of cost estimation is essential. As a general rule, the sample size within a transect should not be less than that indicated by the methods described below. If cost limits the total sampling program, then it is better to eliminate entire transects from the proposed sampling program and retain the quality of the samples taken at the remaining transects, than to sample a larger number of locations in an inadequate manner. Later sections will expand the problems of developing the final program in detail.

A general formula for the optimal sample size for specified precision and accuracy may be derived from the definition of D, the index of precision. D is defined as the ratio of the standard error to the mean,

$$D = \frac{(s^2/n)^{\frac{1}{2}}}{\bar{x}}$$

If the tolerable error of estimation of the mean is 20%, then D = 0.2. For a given value of D,

$$n = \frac{s^2}{D^2\bar{x}^2}$$

This specifies the minimum required sample size to obtain a set precision of estimation of the mean density. If the variance were 100 and the mean 10 for some given species, then for a tolerable error of estimation of 20% of the mean, the required sample size would be n = 25. On the average, a sample of 25 units in the same location would have a standard error of the mean equal to about 20% of the mean. D may also be expressed as the relative error in terms of percentage confidence intervals about the mean. For example, one might specify that the tolerable 95% confidence limits about the mean in future samples should be no more than ± 40% of the mean. D is now 0.4, and the formula is multiplied by the value of Student's t for the desired probability level.

$$n = \frac{t^2\ s^2}{D^2\ \bar{x}^2}$$

For 95% probability level of D, t is about equal to 2.0, so for D = 0.4, the formula reduces to simply

$$n = 25 \frac{s^2}{\bar{x}^2}$$

Zimmerman and Merrel (1976) used a modified formula (Snedecor and Cochran, 1967) to include a specified level of probability of detection of changes in the mean density at any given level. The modified formula is simply

$$n = Y \frac{2\ s^2}{D^2\bar{x}^2}$$

where the value of Y incorporates both the degree to which the mean must change, and the probability of detection of that magnitude of change.

This formulation is more suitable for any study in which changes in density are expected to be important as indicators of pollution. The degree to which the formula increases the recommended sample size over that in the original formula depends on the magnitude of change and on the desired probability of detection. For example, if the variance were 100, the mean 10, the magnitude of changes to be detected ± 40%, and the probability of detection 95%, the required sample size would be 162. The original formula for estimation of the mean ± 40% specifies 25 as the sample size. A probability of 80% of detection of a 40% change in the mean reduces the recommended sample size to 98. In most cases, a smaller sample size will have to be used for practical limitations, and larger changes in the mean, or lower probability of detection, will have to be substituted. An excellent example of the use of procedures for determining sample size is the study of Livingston et al. (1976).

REPLICATION IN SPACE

The previous sections have discussed the aspects of the total sampling program which relate to sampling in a given part of the sampling locations, the transect. The transect, as mentioned in the introduction, is a single part of a location which varies between parts. To be certain that the sampling area is adequately characterized, at least one replicate transect must be taken. The exact number of replicates depends on the likelihood that the sampling location will be internally variable between transects. If the sampling area is extensive, more replicates will probably be needed. Each transect is a complete sampling program as specified by the methods described in earlier sections, with a chosen sample size, sample unit, and stratification. The sample size and sample unit area both affect the number and type of organisms returned in the sample, and should not be changed between transects of the same dimensions. The stratification of the transects should be the same, since the location was defined as at least superficially homogeneous internally. If necessary, the transects could be stratified differently. If so, then the strata probably could not be compared between transects, but the overall transect means could be compared. If strata are selected by biotic discontinuities, which are located in the same relative positions but at different absolute heights in different transects, a comparison of these strata between transects would be valid even if the height intervals differed.

Since the sampling location has been selected on the basis of apparent homogeneity, one might expect that two transects would be sufficient. In some cases, large scale differences can occur even though not superficially obvious, and more transects would be necessary to characterize this scale of variation. The most comprehensive programs rarely have more than 4-5 transects in a given location, and in most cases fewer are adequate.

Relatively few studies have examined the variability between sampling locations, probably because of the extensive sampling program required. Each site would be sampled at two or more transects, and practical limitations would usually limit the sampling program to not more than two sites of any given type of substrate. At least two sampling locations of each type would be sampled to be certain that the results of sampling at one were not a product of unusual conditions at that location.

The essence of the above comments is that at every level, replication is essential if the conclusions reached from sampling are to be general. A sampling program which examines a single sampling location in great detail cannot confidently be used to state conclusions about that entire category of shoreline types. A single transect is an insufficient basis for definite conclusions concerning the remainder of the sampling area. The development of the final program must be a series of compromises, in which the scales of variation of greatest interest are examined. If it is important to gain information about all of the shoreline types in a diverse region, then the degree of variability within each location may have to be less well characterized in order to have the time and resources available to sample in many locations. The variation between transects of a given location is least likely to be of importance. The variation within a transect is most likely to be important, so that transect sample sizes should rarely be reduced. Variations from one type of shore to another may be of great or little importance, depending on the objectives of the study.

TEMPORAL REPLICATION

In addition to the variation which occurs over distances from centimeters to many meters, the abundance of species may change through time from natural causes. These fluctuations are at least as important as spatial variation in interpretation of possible pollution effects. Many species reproduce seasonally and would normally have large fluctuations in abundance. Single samples at one time would potentially over- or under-estimate the average yearly abundance, and changes in the abundance of species could not be separated from possible normal temporal fluctuations without information on these fluctuations. An absolute minimum frequency of sampling would be twice each year, in order to sample at the probable times of greatest and least density. Many species, however, vary from the usual patterns of spring-summer highs and winter lows, so that four times per year is preferable. At each time of sampling, the program is repeated in its entirety if possible.

A related problem is error resulting from short-term variability in apparent abundance, particularly diel periodicity. Many organisms follow diel patterns of activity and are more easily observed during specific parts of the day or night. While it is not practical to sample at all times of day for every sampling run, at least one sample or series of samples should include a check on variation in the recovery of species at different times of the day. In some cases, the time of sampling may have to be changed to accommodate the diel behavior patterns of potentially important species.

Finally, the major characteristic of the intertidal is simply the alternating exposure and immersion of the substrate. At high tide, one can always expect that a number of motile species not present at low tide will be an active part of the community. Since sampling is largely restricted to low tide, this regular alteration of the observable community will seldom be quantifiable. A number of studies have qualitatively examined the possibility that major members of the community are absent during the sampling, by seining or other means of qualitatively sampling demersal or mobile epibenthic organisms.

SUBSAMPLING

If a large sample unit size has been selected, it is sometimes impossible to sample the entire unit. Generally, a large unit will have been selected because it either yields a sufficient number of the species present, or because it has the property of reducing the variance. In the former case, it is the size of the total unit which controls the number of species returned by the unit, so that only by sampling the entire unit can one recover the desired number of species. If the unit was selected because it reduced the variance, then in some cases it may be possible to subsample the unit and retain most of the effectiveness of the unit. Subsampling consists of selecting a random subset of equal-area portions of the unit. If the sample unit can be divided into equal areas or equal volumes (in the case of cores), totalling M possible subunits, a random selection of m of the M subunits is made, and the sampling retrieval of organisms proceeds only within the m subunits. A hazard of subsampling is that if the subunit areas correspond to the scale of patchiness over short distances, then the variance associated with that patchiness will be reflected in the estimate of the variance between primary sample units which is derived from the information on the subunits. For maximum retention of the effectiveness of the sample unit, subunit areas should be selected which are different from the areas of those units in the preliminary sample survey which had high variances. In some cases this is unavoidable, and a loss of effectiveness of the primary sample unit must be accepted.

Estimation of the means and variances of primary sample units is as follows:

1. To find an estimate of the mean of subunits within a single primary sample unit, calculate the mean per subunit using the usual method of estimation of a mean.

2. The variance of the subunit mean within the sample unit is calculated in the usual manner for estimation of the variance of a random sample, with m-1 degrees of freedom.

3. The simplest estimate of the mean per subunit of all primary units, which is the mean per subunit for the sample, is

$$\bar{\bar{Y}} = 1/n\ (\bar{y}_1 + \bar{y}_2 + \dots + \bar{y}_n)$$

   where $\bar{y}_i$ is the mean per subunit within the primary sample unit i, and there are a total of n primary sample units in the sample. The estimate requires that the estimate of the mean per subunit within each primary unit be unbiased, but it does not require that the subunit sample size be constant for all units.

4. The variance of the mean per subunit for the entire sample is given by

$$v(\bar{\bar{y}}) = \frac{1-n/N}{n}\ s_1^2 + \frac{1-m/M}{mn}\ s_2^2$$

   where s_1^2 = the variance among primary units of the mean per subsample unit, and s_2^2 = the variance among subunits of the mean per subunit:

$$s_1^2 = \sum_{i}^{n} \frac{(\bar{y}i - \bar{\bar{y}})^2}{n-1} \qquad s_2^2 = \sum_{i}^{n} \sum_{j}^{m} \frac{(yij - \bar{y}i)^2}{n(m-1)}$$

   where y_{ij} is the value of the j^{th} subunit in the i^{th} unit, $\bar{y}_i$ is the mean of the subunits in the i^{th} unit, and $\bar{\bar{y}}$ is the mean per sample unit over all units, given above.

Further details of the use of subsampling, including methods for selection of the optimum value of m, and use of subunits of unequal size, may be found in Cochran (1977), Venrick (1971) and McAlice (1971).

LEVELLING, SURVEYING AND SAMPLING POINT LOCATION METHODS

For locating sampling point heights in any type of sampling design, and for stratification of transects, the tidal elevation of the various parts of the site or transect must be determined and both the transect or sampling area and specific places inside it must be marked, permanently in most cases.

Site location and transect establishment.

Criteria for selection of sampling areas or sites will reflect the requirements of the particular program and should be well established early.

In pollution related studies sites will be selected for various reasons, such as: because they represent typical, unaltered examples of prevalent habitat types in the region; because of the possibility or existence of degrees of pollution impact at specific places regardless of habitat type; or because they have been determined to be of ecological importance and must be monitored for any signs of alteration or to determine the effectiveness of pollution abatement efforts, or to follow events before, during and after episodes of chronic pollution at sites not identified and studied previously.

Site selection will therefore be determined by program objectives; the variability of the site and the sampling design to be used will determine the extent of the alongshore distance or width of the transect. At the site, the general location for the transect is selected in a relatively homogenous and representative area, and a reference line, identifying the top of the transect is established. If the area is sufficiently homogenous, a reference line longer than the transect width may be laid out and by the use of a randomly picked number, a starting point for the segment to be used as the transect may be selected. Reference lines should be placed at the extreme upper reach of waves at high tide if possible, to minimize damage to permanent markers. On rocky shores at the base of cliffs, a point on the extreme backshore reach of the intertidal bench should be used.

Levelling, stratifying and marking transects.

By the use of a transit or a sighting compass and temporary range poles, the reference line should be oriented as parallel as possible to the low tide waterline; this will probably also be parallel to the contours of other tidal levels and result in approximately rectangular strata within the transect.

All sampling points on a shore must be referred to a permanent reference point of known tidal height, located by a marker in the supratidal just above the reference line or on the reference line. The height of the reference point itself is preferably determined by comparing its level to a U. S. Coast and Geodetic Survey marker, or a secondary monument established from USGS levels, in the vicinity. Alternatively, its level may be compared to water levels at the predicted time of low tide on calm days, with the time and level of the tide corrected for the site from corrections given in the tide tables issued by the U. S. National Ocean Survey. If a USGS marker is to be used and one can be found near the site, this should be done before transect establishment and the number designation of the marker sent to the Survey, with a request for the height of the USGS marker with reference to mean sea level and a correction for chart datum (local 0.0 tidal level). A standard surveyor's tripod mounted transit, preferably of the self-levelling type, is the best instrument for bringing the level of a distant benchmark down to the permanent marker at the top of a site. Bring the level down to the site with transit and levelling rod and then back to the benchmark, to adjust for accumulated error.

The permanent reference point for beaches and flats can be marked by driving a metal rod or pipe several feet into the top of the beach, nearly level with the surface, and the top of the marker used as the reference level. A metal or fiberglass fence post, driven in deeply with a capped length of iron pipe of a diameter sufficient to slip easily over the post, can be placed a short distance from the reference marker as a witness post for easy relocation of the marker should it become obscured or buried by debris.

On rocky shores, markers must be affixed into the rock by some means suitable to the rock hardness and friability. Marks made on the rock with picks or chisels are usually initially obvious but under coastal conditions weather into obsurity remarkably fast. Permanent markers should be installed in holes drilled with star drills, or masonry drills and a portable self powered electric drill, and plastic or lead lag shields used to secure stainless steel or bronze screws and washers in the holes. Epoxy putty which sets under water, or concrete, may be used as an aid in securing the lag shields in the holes. In some soft rocks which do not fracture easily, a powder actuated stud gun can be used to shoot masonry studs into the rock, or hardened concrete or masonry nails may be hand driven, with a perforated metal disc threaded on to the stud or nail and held by the head. In very hard, fracturable rock like basalt, no holes can be easily drilled nor can studs or nails be driven in. A larger hole is made with a heavy chisel and hammer,and cement or epoxy putty is used to fix a lag shield or large bolt in the rough depression. All of the above methods are also used to place subsidiary markers, such as those for tidal levels or previously used quadrat locations, if they are to be permanently marked. Intertidal markers on any beach type will require maintenance. Avoid placing markers on rocks in pre-existing cracks; they are places of previous weathering and weakness.

In locations where no bench mark is available, the height of a permanent reference point is determined relative to water level at the site. Tidal heights vary only slightly over long distances and the tide tables have corrections for many points along coastlines and estuaries, adjusting both time and tide height for these points. The time and level correction for the place nearest the study site may be used. If there is reason to believe that this is insufficient, a site correction should be determined before transect levelling proceeds. Local tide staffs or tide gauges are invaluable for this but are not likely to be available at remote locations. On calm days with minimal wind and surf, tide observations on rocky shores can be made in meander channels freely connected to the sea where water level is the least affected by waves. On sandy beaches of the open coast, a levelling rod is hand held in shallow water, or a temporary tide staff is erected by sinking a marked rod into the sand just below the level of the low tide to be used. The midpoint between the crests and troughs of waves where the rod is held or fixed is estimated and this is used as tide level.

Using either natural landmarks on rocky shores or the rod method described for sandy shores and flats, water line observations are started about half an hour before the predicted time of low tide for the nearest places with predictions available, and observations continued for the same period after the time. The actual time, and on rocky shores or fixed staffs, the level, of the lowest point during the observations is the time and level of the low tide for that place. This provides a specific low tide time correction for the site, which is then verified by repeating the procedure at several other low tides at the site. Height corrections are seldom needed, and require that the type of observations described be repeated for a set of landmarks or a temporary tide staff at high and low levels over a longer period. Using the predicted tide time and level and any necessary site correction, water level at the time of a specific low tide is now used to determine the height of the reference point. Transit or hand level methods are used to compare the water level reached under calm conditions at the time of low tide with the reference point on several low tide periods. This is most conveniently done by placing the tripod or hand level position at a midpoint on the beach and then observing the levelling rod starting just before the predicted time of the lowest point, to determine the height of the instrument relative to water level. The instrument is then turned in place and the instrument height determined relative to the reference point, yielding the height of the reference point relative to the low tide water level.

Much of the required levelling within the intertidal for laying out transects and heights within them is adequately done with a surveyor's hand level, either the simpler Locke type or the Abney Level-inclinometer type, held against an extra levelling rod at eye level to avoid fatigue and uneven footing effects. The distances involved in intertidal levelling can be kept down to short stretches and the rapidity of hand level operation permits accuracy to be quickly checked by repeating the levelling. A sighting, or Brunton, compass will keep the lines sufficiently straight, if the eye will not suffice over short distances. On rocky shores, the potential greater accuracy of a tripod mounted transit is offset by the much greater time required to set up and lay out a transect, because of the small horizontal irregularities and great vertical changes over small distances as well as the difficulty of moving the heavier, larger instrument about. On the smooth surface of flats and beaches, the greater horizontal distances and the efficiency of levelling many points from one position make the transit the preferred instrument.

For all types of levelling, the horizontal distances between the points of determined height are measured to reconstruct the slope and horizontal distances between the known vertical intervals and so that a more accurate estimate of the area of each stratum in the transect can be made.

Once the reference point level is determined, the transect is laid out using a levelling rod and a transit or surveyor's hand level to establish a center line or two side boundary lines for the transect, perpendicular to the top reference line. Regular vertical increments, preferably of one foot (0.3 m), to be used as strata limits, are marked along the lines with stakes in beaches or by the rock marking methods described. If permanent markers are not installed, the levels on rocky shores may be easily relocated by using the reference marker to re-level the transect every sampling period, but profiles of rocky shores are stable and permanent markers once placed will subsequently save time. On open coast sand beaches, strong seasonal changes in profiles are a normal annual event and the transect must be re-levelled and strata width remeasured at every re-sampling period. Temporary, wooden stakes are adequate to mark the vertical contour intervals on beaches. Flats are more stable and while some long term changes are to be expected, those in very protected situations may be permanently marked with deeply driven metal rods which should not protrude above the flat higher than needed for relocation. Permanently marked tide flat levels can be quickly checked for change at each sampling period and complete re-levelling avoided.

Despite the known effects of wave exposure on the vertical limits of intertidal organisms on all types of shores, the only objective physical reference available for comparing levels on shores at different sites or at different times, is standard tidal height, especially in studies emphasizing spatial and temporal variations and their causes. For this reason, the vertical increments bounding strata in transects should be regular increments related to the tidal reference level of 0.0 rather than the height of the reference point itself. This will result in a standard stratification which will be identical at all sites, with, for example, a stratum bounded by the +2 ft. (+0.6 m) and the +1 ft. (+0.3m) above MLLW contour levels present in each transect. Strata bounded by any arbitrary height intervals are adequate for work within a specific transect if no comparisons are to be made to conditions elsewhere.

Open coast beaches and many rocky shores (disregarding microrelief) have uniform slopes for long horizontal distances along the shore, resulting in parallel tidal contours. In these cases, stratification is easily achieved at the time of sampling by stretching a rope or surveyor's tape in a straight line across the transect width, between the markers for a given tidal height. On some rocky shores, and commonly on tidal flats, the contours at a given tidal height may meander significantly within the width of the transect. Stratification of tidal flats, especially in transects on the order of 300 ft. or 100 meters wide, requires the establishment of a number of points of the same height at each vertical interval rather than only two on each side of the transect. Depending on the degree of irregularity, several points of the same height should be found between the two located on the side boundaries. These should be marked with tall temporary stakes, and a sighting compass, transit or the eye used to establish a single straight line, approximating their average position, across the transect and perpendicular to the sides and the end positions staked on the sides. A strongly curved stratum boundary would make the use of rectangular coordinates to randomly locate sample points within the stratum very difficult.

Once the sides of the transect or a center line are located by marking the strata boundaries at equal vertical intervals, the location of the sample points requires that the dimensions of each stratum be measured. These measurements will also permit the slope of the transect to be reconstructed accurately. Because of variations in degree of slope down the beach to waterline, the depth of strata will vary within the transect; upper strata and lower strata will be narrower than those at mid-tide level because of the characteristic mid-tidal action of waves. Both measurements and location of random points are done with surveyor's tapes, preferably plastic impregnated cloth or fiberglass tapes which neither stretch nor corrode when wet with sea water. Metal tapes are quickly destroyed on sandy and rocky shores by abrasion and corrosion.

The linear dimensions of the strata are also used to calculate their actual area, and this is then immediately used in the field to make the final allocation of samples units to each stratum. Location of random points is easily achieved by laying a tape down the longest boundary of a stratum, and using it to locate one of the coordinates from which the second is located by measuring with another tape or pacing at right angles to the first tape. Only the second tape need be moved for each pair of coordinates, and the first tape can be left in place and used for the stratum above and below it, if it is laid on the vertical interval line between strata. For field use, the estimated total number of sample units to be used in the transect is used to calculate the amount of area represented by one core sample or quadrat, for the entire transect, or for individual strata if sample size has been independently estimated for each stratum planned. This may now be used to allocate samples to strata on the basis of their actual area calculated from the measurements of their dimensions, as described elsewhere. Basically, the area to be represented by one sample unit is divided into the total area of each stratum to yield the number of points to be sampled. The area of the usual quadrat or core used is sufficiently close to one square foot or 0.1 square meter in transects on the order of 100 ft. or 30 m wide, that the foot markings or the 0.1 meter intervals on standard surveyors tapes can be directly used to locate points. The maximum length and width of each stratum in feet or 0.1 m intervals are used as the limits when selecting the coordinates for random point location from a random number table.

On intertidal sand and mud flats, and on some rocky intertidal benches, certain strata, particularly the lower ones will be long and narrow in their dimensions across the width of the transect because of the relatively steep slope of lower intertidal areas. This arrangement maximizes the possibility of the rare event of the chance clustering of the random points entirely within one region or one end of the stratum. Dividing very narrow strata down the middle and randomizing within each half minimizes this chance, with half the sample unit allocation being assigned to each side. Alternatively, if a set of randomly drawn coordinate pairs clusters the sample points closely, the entire set may be rejected and another entire set of random pairs drawn but do not attempt to "adjust" the original set. Extensive use of this practice, which rests on a subjective judgment, will result not in randomization, but at best excess spacing of samples to some unknown but biased degree.

Retention of the coordinates used in randomization in each stratum, and serially numbering sample units within the stratum in the order of the use of the coordinates as well as giving each unit a stratum and transect designation will completely locate and identify each individual sample unit.

III. COLLECTING AND PROCESSING SAMPLES

Introduction

The most common approach to sampling of intertidal biota is recovery of actual specimens in a quantitative sample, from which the density, biomass, diversity, and other information, can be estimated. Since such sampling removes a portion of the biota which are present in the field, it may be referred to as destructive sampling, whatever the actual recovery method. An alternative to destructive sampling is the acquisition of similar data in the field using methods which do not disturb the community.

Two major arguments may be presented against destructive sampling and for non-destructive sampling. The first is the fundamental one of attempting to minimize the damage done to an area in the name of science. The second relates to the information gained from such sampling, because repeated sampling of an area could in many instances be biased by the effects of previous sampling. These effects are most noticable when the species sampled are long-lived, with poor recruitment capability, and low density. A prime example is the removal of predatory starfish, which may have low recruitment capacity compensated for by a long lifespan, and which have a major effect on the community as top carnivores. Other sensitive species which may operate as keystone species in the community are urchins and predatory gastropods. Removal of such species from an area, or any substantial reduction in density, can alter the rest of the community in such a manner that change due to other events may be undetectable.

Species which have a high reproductive capacity may also be sampled under circumstances which bias results. An example might be removal of a set of quadrats primarily containing barnacles. The individuals present in a given position may represent the results of several years changing conditions, in a succession from early colonizing species, to species which overgrow others, and finally to especially large individuals of those species which reach an escape in size from their predators. Several years might elapse before the patch created by the destructive sampling stabilizes in a form similar to that which was removed. During this time, it would be difficult to come to conclusions about the seasonal succession of species, recruitment of new individuals, or other problems near the disturbed area. While the sequence of succession varies with the community, evidence suggests that returns to the original intertidal community may often require years. Monitoring studies of a single area run the risk of distorting their own conclusions. There are conditions, however, under which destructive sampling is not likely to affect future sampling.

Unconsolidated substrates are characterized by frequent or continual natural alteration of the substrate. High-energy environments are periodically shifted by increased wave energy and erosion of sediment, or decreased energy and deposition. Low-energy environments usually experience continual deposition, and in the case of mudflats often experience alteration of channels, bioturbation, and other substrate disturbances. The fauna of sedimentary shores are usually mobile to the extent that they can reposition themselves in the sediment, if not freely move around. Minor disturbances such as coring will be quickly filled in by the surrounding organisms, so that the effects of sampling are undetectable in a short time. The reduction of populations of certain species which are slow to replace individuals can potentially affect either a local density, or alter the community composition. It is not likely that the typical sampling program would noticeably affect communities in sedimentary environments.

Rocky shores, in contrast, have a stable substrate. Communities differ greatly in their ability to fill in gaps or patches caused by sampling. Mussels, for example, can reposition to fill a small patch. Barnacles must recruit to replace a patch of removed individuals. In general, a more complex assemblage of species will require a longer period to re-establish the community pattern which was observed at the time of removal, than locations where a monoculture dominates. Even monocultures, as stated earlier, may be the result of a succession rather than an immediately reached pattern. Sampling should never assume that the effects of previous sampling are negligible, and previous quadrat positions should be marked and not be used again except to observe the sequence of succession.

Non-destructive sampling is potentially applicable under any circumstances where the species to be sampled are visible, measureable, and unaffected by the sampling procedure used. Most of the sessile macrofauna on rocky shores may be sampled without removal, as is frequently done for macroalgae. Quadrats are placed in the usual manner, and individuals counted and measured in situ for some suitable dimension which can be converted into an estimate of biomass. In some cases, it may be possible to sample some species in sand, mud, or gravel sediments, provided that either some superficial sign of individuals is available (such as openings to burrows), or the individuals may be recovered and replaced without damage (such as cockles, sand dollars, and other shallowly buried species).

Non-destructive sampling is inadequate to sample small and mobile species. When diversity estimation is the major goal of the study, some destructive sampling will be essential. Non-destructive sampling is particularly suited to studies of the species which contribute a majority of the biomass in many rocky intertidal communities, including barnacles, mussels, starfish, urchins, limpets, tubeworms, macrophytes, anemones, and others. When non-destructive sampling is used to estimate the size of populations of these species, a minimum of destructive sampling precedes, to establish the conversion ratios from numbers and linear size to biomass.

An important advantage of non-destructive sampling is that several of the possible methods, particularly photography, allow very large samples to be taken. These techniques have useful applications when the time in the laboratory is unlimited, but that in the field is very limited by seasonal weather, number of low tides in the time available, inaccessible sampling locations, or briefness of the exposure of lower areas in the intertidal.

Other methods used in non-destructive sampling involve field measurements which may take as much or more time than sampling by removal, and their accuracy is usually low.

Destructive sampling methods for sedimentary shores.

Coring Devices

A corer is any device which removes a quantitative volume of sediment. Most corers are designed to do so without excavation of the interior of the corer. In moderately wet sediments, a small corer will usually provide enough frictional resistance to the core (the sediment trapped in the tube) to keep the core from falling out. Larger corers may be capped or otherwise sealed at the upper end so that the vacuum effect at the top of the core helps to hold the core in the tube when the corer is removed. In very wet sediment, unless the pressure at the top and bottom of the corer is equalized, suction at the bottom of the corer will make it very difficult to remove a core intact. Loose, dry, or large-particle sediment is not retained well by corers and must generally be excavated from the interior of the unit. In compacted sand, it is often helpful to have the coring tube mounted on some sort of handle so that more pressure may be brought to bear on the corer.

An assumption underlying the use of coring devices is that the insertion of the corer does not disturb the sediment and the organisms sampled. The assumption is always violated near the walls of the corer, where frictional resistance of the sediment and corer wall distorts the sediment during both placement and removal of the corer. The walls of corers should be as thin as possible for this reason, within the limits of the required strength of the corer. Small corers will have a proportionally larger area subject to distortion than larger corers.

During placement of corers, there is a tendency for the sediment already in the corer to act as a plug which forces the sediment aside. This increases as the core lengthens and frictional forces increase. Loose, wet sediment is pushed aside after relatively short distances, while it takes longer to displace well-compacted sediment. Wet mud or sand must either be sampled by using a larger corer and rotating or rocking the corer into place, by sampling with a series of successively deeper cores in the same position, or by countering the frictional resistance of the core in some manner. Some corers produce a mild vacuum above the core which counters the friction of the core and helps to drive the corer in deeper. Rocking or rotating a large corer into position can be used to take a fairly deep core, but caution must be taken not to disturb mobile organisms which may then evade the corer. A vacuum assisted corer, or one which takes a successively deeper series of cores, may be more reliable in these applications.

Virtually any thin-walled strong material can be used to construct corers. Metal is acceptable if corrosion is avoided, as corrosion increases the frictional resistance of the metal surface. PVC or other plastics are excellent for smaller corers, but are expensive for larger diameters. Small

PVC tubes are slick enough so that the upper ends can usually be sealed briefly by hand or stopper during retrieval of the core. The core is then quickly released on removal of the stopper. Corers of any material or design should be tested on sediment of particle size and compaction similar to that at the actual sampling location, as a seemingly small difference in the sediment texture can mean the difference between a working and a useless corer.

Many pollution surveys combine sampling for chemical and biological analyses to increase efficiency of field efforts. The choice of the material used to make corers may depend upon whether more emphasis is to be placed on organic or metallic pollutants. The requirements for both chemical non-contamination of the sample and effectiveness in securing the biological sample should be considered early in the design phase of the study. For example, aluminum is seldom of importance as a metallic pollutant in marine environments and aluminum tubing has a number of desirable physical characteristics as corer material while stainless steel which has even better physical properties may add copper, chromium or other metallic contaminants to the sample.

The following describes a selection of sampling corers which illustrate corer designs. Many other possibilities exist, and the individual worker may choose to develop a more convenient design.

1. The simplest corer is a tube of sufficient length to penetrate the required distance and still leave room for a good handhold. Small corers used in firm sediment can easily be taken by hand pressure alone, and the simplicity of such corers has the advantage of rapid preparation and replacement of corers. The leading edge of the tube should be beveled on the outside to cut through the sediment without forcing sediment into the tube. Gentle pounding can be used to drive in tubes if the sampled species are unable to evade the disturbance.

2. A simple variation on the above design is to mount the corer on a handle. The usual handle is a simple T shape. The handle is hollow, and open to the top of the coring tube and to a small hole at the top of the handle which is used to seal off the coring tube during removal of the corer from the sediment. A crossbar at the top of the coring tube allows foot pressure to be applied to a strong part of the device rather than the more fragile tube itself. Some designs permit interchangeable tubes of different size, or removal of the handle from the tube for easier emptying of the corer.

3. A more complex design is suited for sampling in very wet sediment. Two corers are placed side-by-side, one acting as the corer and the other to equalize pressure. The first corer is placed into the sediment and sealed at the upper end. The second is forced into the sediment as a thin rod, the lower end capped by a plug connected to a rod inside the tube. When the second corer (the vent) has reached the same depth as the first, the inner rod is used to force the plug

out, so that the vent is now a hollow tube extending from the surface to the bottom of the first core. The first corer may now be withdrawn without collapse of the walls of the hole, and without suction, because the vent has equalized the pressure at the top and the bottom of the first corer. After removal of the first corer, a longer tube can be inserted into the same hole if desired. The sample is extruded from the corer by a fitted plunger, so that it can be sectioned if desired. The corer barrel acts as a resonator, so that frictional binding of the core to the tube can be heard as a change in the sound of the corer, and the corer is then stopped, removed, and a longer corer used to continue to the maximum depth.

4. Satisfactory simple coring tubes may be made of lengths of aluminum irrigation pipe, which is strong and has very smooth, thin walls. Corrosion is easily prevented in this pipe by cleaning the corer after use. Corers of this tubing, varying in diameter from three inches (7.5 cm.) to six inches (15 cm), have been used sucessfully in a variety of sandy and muddy sediments. The corers are constructed from a two foot (0.6 m) to three foot (0.9 m) length of tubing, with a plate welded across one end to close it. A braced handle of small diameter thick walled aluminum pipe is welded across the plate and a small hole in the plate near the handle permits air to escape when the tube in pushed into the sediment. The tube is of sufficient length so that the operator can insert it into the sediment from a standing position, bringing body weight to bear on the handle by leaning forward. The hole in the top plate is closed by thumb pressure while withdrawing the corer from the sediment to aid in retention of the core. Plastic tape or paint on the outside of the barrel marks the depth the corer is to be inserted into the sediment.

   Corers as large as those described above can be used to their full length, but may lose the core if they are pulled up vertically. The cores may slip out of the large tube because there is insufficient suction and frictional resistance to overcome the pressure difference from surface to the end of the corer. This difficulty can be overcome very simply. The corer is inserted as vertically as possible, but pushed over horizontally before being withdrawn. With the air hole closed, the corer is slowly pushed sideways until the sediment breaks upward and the end is near the surface and can be grasped. The corer is then pulled free of the hole nearly horizontally. In packed sand, or stiff mud, a small shovel can be used to dig down near the end of the corer, equalizing the pressure and permitting it to be more easily tilted sideways for removal.

5. A coring type sediment sampler with a barrel diameter of about 7.5 inches (18.5 cm), and a closing plate is described by the Finnish IBP-PM group (1969), illustrating some of the possibilities for the use of a large sampler based on the corer design.

6. Smaller macrofauna and meiofauna of intertidal sedimentary shores can be easily sampled with small piston corers made from 50 ml. or larger plastic disposable syringes by cutting off the bottom, or needle end of the barrel. The resulting tube is strong and thin-walled, and the plunger is used to both aid in insertion of the corer and to retain suction on the core as the corer is withdrawn. The cores are kept intact, and may be slowly extruded and sectioned for vertical distribution studies. The relative cheapness of plastic syringes permits many corers to be available for storing cores temporarily until processing.

7. Samples for the analysis of sediment characteristics are often taken separately from those for organisms. Thin walled plastic tube, used as corer liners in deep sea coring devices, in short lengths makes useful sediment sample corers. The most convenient sizes of this tubing is from one to two inches in diameter (2.5 to 5 cm).

8. If deeper, large macrofauna must be sampled and the substrate does not permit a clean hole to be dug because of collapse of the wall, a large sheet metal caisson can be used. This is any large cylindrical or square container with straight sides and open ends, with handles attached to the sides at one end so that it can be pulled out after use. A caisson must be large to encompass enough area for an adequate sample of larger, rarer burrowers like bivalves and thallasinid shrimps. It must also be large enough in the cross-sectional dimension to permit the operator to work within it. A caisson 0.3 m to 0.5 m in diameter and 0.5 m or more long will meet both requirements. The caisson is sunk into the substrate as far as it will conveniently go to start the sampling, and a trowel or scoop is used to dig out the sediment inside. The decrease in inside friction will gradually allow the caisson to be sunk to the required depth as excavation proceeds. This work should be done at a time when the flat or beach has had an opportunity to drain at low tide, or the depth in the sediment at which the caisson will fill with water faster than it can be bailed will be too high for a deep sample to be taken. The sediment and water within the caisson must be sieved in the field; a coarse sieve of a mesh size of 0.5 to 1.5 cm is usually adequate. This is a laborious procedure and the time requirements will preclude many samples from being taken unless sufficient personnel are available.

   In beaches of fine sand, the drained sand at low tide is often sufficiently compacted so that a hole with firm walls can be dug. The area is measured out on the surface and carefully dug out with shovels, maintaining the wall dimensions as well as a standard depth. In quiet embayments, diver-operated suction pumps have been used at high tide over flats to sample a standard area to depth, within a quadrat frame or ring, with the suspended material being passed through a large hose to a sieve or mesh bag. Underwater holes do not collapse and the relative crudeness of the method is offset by its independence from the tides and its relative speed compared to hand digging holes at low tide, and sieving the excavated sediment.

Handling core samples in the field

Transects on both beaches and flats will require a relatively large number of core samples, of heavy wet sediment, which must be moved about. In order to use the available time at low tide for taking the cores, it is impractical to sieve each core as it is taken. A practical method is as follows. First, the beach or flat is surveyed and levelled, and the random or systematic position of every core to be taken marked with a light lath or marker tall enough to be easily seen. The coring may start before the entire beach is surveyed if there is sufficient personnel.

For efficient use of the available time at low tide a team of two persons is required for core sampling. One person operates the coring tube, while another holds open heavy plastic bags large enough to hold the core sample and also fills out the remaining details on prepared labels. The corer operator puts the end of the corer into the bag, releases the air hole and slips the core out. The other operator inserts the label, adds formalin if it is to be used, and ties the bag shut securely. The bagged core samples are left next to the places they are taken from until at least the lower strata of a transect are completed. The surveying and sample point location team can begin to gather up the bags, carrying them in buckets to the top of the beach, or using a light cart with bicycle wheels, transfer them to a staging area.

Beach and tide flat sediments drain at low tide and there is virtual immobilization of most of the species within the cores. If the cores are kept out of the sun, most forms will survive for long periods in bagged cores. Such cores may also be transported to the laboratory and stored under refrigeration for more than 24 hours in good condition.

If sieving is to be done on the beach, it may be done at high tide, between low tide sampling work. The bagged samples may be left just at waterline to keep them at sea temperature. Beach sieving has the advantage of an abundant supply of water, as does sieving on a dock. Samples accumulated at the top of the beach may also be picked up by small boat at low tide to minimize carrying distances.

The above presupposes that sieving will be done while the organisms are in good condition, and that it can be accomplished before degredation. Time constraints may require that the sampling program complete a large amount of coring and produce too many samples to sieve within 24 hours after they are taken. In this case, or by choice, the entire core sample may be preserved intact, with formalin. For a given sieve mesh, more and smaller animals are retained in some taxonomic groups, when sieving is done on preserved material. Entire cores, 15 cm. in diameter and 20 cm. long can be sucessfully preserved inside thick walled plastic bags, and after securely tying the bags closed, the samples may be temporarily stored within the bags until they are transported to the laboratory for sieving. For success by this method, adequate amounts of formalin must be added and mixed into the sediment sufficiently. The latter is relatively easy in sand, and more difficult in mud. For fine sand, a pore space of approximately 25% of the volume may be taken as the water volume within the core. The core volume is calculated, an estimate made of the pore space, and sufficient concentrated formalin solution added so that when diluted by the pore water, the result will be a 10% formalin solution. Do not attempt to add 10% formalin to cores; the result will be formalin of a greater dilution and a large amount of fluid standing above the sediment in the container, adding to the weight and hazard of handling. After tying the bag closed, the bag is shaken to mix the contents well. If the plastic bags develop leaks, they may be slipped into a second bag to contain the leakage.

Much of the hazard and difficulty of handling concentrated formalin in the field can be obviated by the use of a portable garden sprayer to dispense the formalin. A plastic or metal sprayer, with built-in pressure pump and shoulder strap is filled with two gallons (7.6 liters) of concentrated formalin and taken to the field closed and unpressurized. When needed, the tank is pumped, and the formalin dispensed through the application pipe with the atomizer removed. The core is placed into the heavy-duty plastic bag, which is left open, the metal pipe is thrust into the middle of the core and the valve opened, squirting formalin under pressure into the sediment and mixing it. A previous test of the amount of formalin delivered during a time count is adequate to assure that sufficient is used in each bag. This method permits formalin to be used in the field without any pouring or spilling and the formalin under pressure is discharged only within the sediment and so does not splash out of the bag. After formalin is added to the bag, it is tied closed, and shaken to further mix the contents. For further sealing, electricians plastic tape may be tightly wrapped around the tied and twisted neck of the bag.

Separating organisms from sediments

Sieving methods

The separation of the organisms contained within sediment samples must be done with the same degree of care and precision used in sample program planning and execution. If the program is to quantify both the meiofaunal component as well as that conventionally referred to as macrofauna, the sample will have to be either double from the outset or split for processing because of the different requirements. The macrofauna is conveniently separated from the sediment by passing the sample through a mesh that retains the organisms larger than the mesh size and passes the sediment and all smaller biota through the mesh. Sieving is then a secondary subsampling procedure for the primary sample and should be so treated.

The efficiency of this sampling is a function of the size and precision of the sieve mesh as well as its geometry. Today there are both conventional meshes of woven metal or other flexible material such as nylon available and also sheets of plastic materials with precision holes of different sizes, spacing and density. All of the geometric parameters of the effective sieve mesh determine retention properties and these should not be ignored, as they affect different species according to their own shape.

Continual use gradually deforms the mesh of a sieve by impact, clogging and many other hazards of use; it is therefore important to start with a good sieve and inspect its condition during the course of a long program. Cheap substitutes for precision meshes, such as window screening may be a necessity of the moment in emergencies, but have great variability of mesh dimensions, distort readily and have a short life. The probability of an organism of a given shape and size passing through a round or square aperture of the same maximum dimension differs slightly but probabilities for both differ greatly from that for a rectangular mesh. Mesh geometry and its effect on capture efficiency is discussed at length in the literature on plankton nets.

For almost all intertidal work, sieves with a mesh size of 0.5 mm diameter for round holes or internal side dimension for square woven mesh is adequate to retain most of the macroorganisms. A safer method of sieve selection for an unknown biota is to test sieves of different mesh size for the number of species they retain, when the preliminary sampling is done, by nesting sieves of different mesh. The advantage of using the largest satisfactory mesh possible is the reduction in sieving time that results with using larger meshes. However, DeBovee et al. (1974) point out that the assumption that a given sieve mesh size will be adequate to retain a constant number of species in a given fauna may be violated if there are major seasonal changes in the size of organisms. This is exactly the effect of seasonality of reproduction, and one of the objectives of pollution related work is to identify the cause of such variability. The size class at which juveniles enter the sample is obviously controlled by the mesh size used. If a sieve mesh larger than 0.5 mm is used, periodic checks on the degree of retention should be made to ensure that approximately the same percentage of total species is retained throughout the study. Recruitment studies may require two scales of sampling and two sieving routines, if the requirements for adult sampling prove very different from those for juveniles (George, 1964). The retention differences for a given sieve mesh size between live and preserved animals of the same species has already been mentioned.

The most readily available sieves manufactured with precision meshes are the standard geological sieves made principally for particle size analysis and available in a graded series of mesh sizes. For strength, durability and resistance to corrosion, the mesh of the sieve should be made of stainless steel cloth in a bronze, brass or stainless steel body. Meshes made of brass or bronze distort more quickly and special care must be used for the prevention of corrosion. Both standard cylindrical 8 inch (20 cm) and 12 inch (30 cm) diameter geological sieves are available; the larger size significantly reduces sieving time. Geological sieves nest tightly and a large mesh sieve, with a 0.5 to 1 cm mesh size can be nested over 1 mm and 0.5 mm mesh size sieves to catch coarse debris and rock fragments which tend to clog the finer mesh. Sediments containing gravel may require an even coarser top sieve to screen off the rocks and prevent them from damaging smaller animals caught on the finer screens.

Precision mesh metal screening is available commercially and sieves can be made of a size, shape and mesh suitable for particular uses. Metal screens should be carefully braised or soldered into the frame so that there is no space at the junction of screen and frame where small animals can become wedged; even some commercial geological sieves may need to be sealed at this place. Sieves can also be made of precision nylon monofilament cloth, mounted in frames of plastic, for pollution studies where samples must be kept free of heavy metal contamination prior to chemical analysis. Nylon sieves distort readily and care must be taken not to load such sieves with heavy quantities of sediments.

When washing sediments through sieves, do not place sediment samples in a sieve and wash them through with a stream of water from above; this shreds both live and preserved organisms and forces some through the mesh. Sediment samples are either first suspended in a large volume of water which is then flowed through a sieve, or they are placed on the sieve and washed by immersion. Sediments can be manually suspended by gently stirring the sample into a large container of sea water and with continued agitation, pouring the suspension slowly through the sieve set to minimize the force. Mechanical means for washing large numbers of samples are described by Birkett and McIntyre in Holme and McIntyre (1971) and by Pedrick, 1974, who described a non-metallic device suitable for pollution studies.

Manual washing by immersion is done by placing the sample or parts of it in the top of a nested set of sieves or a single sieve, partly immersing the sieves in a bucket or tub of sea water almost to the rim of the sieve and washing the sediment through with a gentle rocking and up-and-down motion.

The material retained on sieves will consist of organisms, debris, coarse sediment, shells and with muddy sediments, small compacted lumps of mud as well as fine particles clinging to all these materials. Do not prolong sieving in an attempt to thoroughly clean the sample. Retain all the material by collecting it to one side of the sieve. Hold the sieve at an angle and dip its bottom into water at sucessively steeper angles until the sample is all against one edge. Pushing the sieve contents across the mesh can damage small, delicate organisms by abrasion, so transfer the sample to storage containers with minimal handling. Use a brush and a fine stream of water directed from the back of the sieve to move the sample over the side of the sieve into the storage container, along with the sample label. Time is saved in field work if the containers have been previously prepared by putting a measured amount of undiluted formalin or other preservative in them. After the sample has been transferred, immediately add more sea water to the final level required for proper dilution and then shake the capped container to mix the contents

The use of nested sieves of graded sizes prevents clogging of the lower finer mesh, but it also results in a type of geometric size sorting of the organisms as well as faster sieving. Keeping sieve fractions separate for each sample may be advantageous if this size fractionation is satisfactory for the organisms of interest, but this is at the expense of at least doubling the number of storage containers to be handled. Sieve separation of organisms by size is affected by the shape of the species and its effectiveness is very different for bivalves and polychaetes for example.

Samples sieved from muddy sediments will still contain fine particles which may later interfere with sorting and greatly increase the time needed. The preserved sample may be more thoroughly washed later by manually washing it on a sieve finer than that used initially to ensure against sample loss. A more automatic washing apparatus will further save time; one is described by Worswick and Barbour (1974).

Separating Organisms from Debris-Flotation and Staining

One of the better rapid methods of separation of small organisms from debris in either field or laboratory is to float the lighter organisms free of heavier debris using a medium of suitably high density. Among the media suggested have been saturated solutions of sugars (Birkeland et al. 1976), calcium chloride (Coleman and Hynes, 1970), magnesium chloride (Hummon, 1974), zinc chloride (Mattheisen, 1960), and carbon tetrachloride (Whitehouse and Lewis, 1966).

Small organisms can be floated free of shell and other debris after collection, but the same essential method may also be applied as a sampling method in the field. Ertl (1970) suggested a device which permitted the retention of a pool of a suitable high-density medium on rough rocky surfaces. Two metal or plastic cylinders are nested, the inner one about 2 cm smaller in diameter and somewhat higher. The space between the two cylinders is filled with Plasticine or some similar pliable material, which when pressed against the rough surface seals the inner cylinder. Ertl then suggested partial filling of the inner cylinder with water and pipette decanting of the scraped organisms. The same device could be used to retain a higher density medium which would float free small mobile organisms which can easily escape normal quadrats.

Flotation methods are especially useful when the difference between the density of the organisms and the debris is great, such as in sand with a high shell content.

A second method for separation of organisms from debris is staining. Williams and Williams (1973) examined the efficiency of many stains in sorting of benthic marine samples, including gentian violet, eosin, fluorescein, malachite green, iodine, fuchsin, methylene blue, rhodamine B, carmine, lignin pink, rose bengal, and Lugol's iodine. Of all of these, they found that rose bengal and Lugol's iodine were the best standard stains. Both are taken up by the animals and the debris, if it was organic in nature, but animals concentrate rose bengal to a darker color than does organic debris, and concentrate Lugol's iodine to a stronger orange color. They also tested the effectiveness of stains which provide a dark background (counterstains) and found that chlorazol black E provided the best counterstain for contrast with the above primary stains. Suggested concentrations for the different stains were 200 mg/liter for xanthene rose bengal, 1 gm/100 ml water of 70% ethanol for chlorazol black E, and 1 gm iodine plus 2 gm potassium iodide per 100 ml for Lugol's iodine. If water solutions are used, the stains should be dissolved in distilled water. Primary stains should be added to benthic samples in a ratio of about one part stain to four parts solid sample and let stand for at least 24 hours. The counterstain is added to cover the drained sample for only 30 seconds, then the sample is rinsed and sorted. The primary stain can be left in the preservative solution for several weeks, but the counterstain leaches out of the sample into any large volume of liquid and restaining would be required.

Destructive Sampling - Trenching and Other Linear Sampling

Trenching provides a quantitative sample consisting of a series of contiguous sample units taken in a line. It can be used only when the consistency of the sediment permits excavation of a sample unit without physical retention of the sediment walls, and is therefore primarily applied to compacted, well-drained sand. The individual units are often rectangular instead of square. There are two major disadvantages of trenching as opposed to standard quadrat sampling. First, the amount of sediment removed is considerable, and sieving must take place at the site. Quantitative sieving of large samples requires considerable effort and manpower. Second, a linear sample does not examine the heterogeneity of the beach parallel to the waterline. Finally, a long, rectangular sample unit tends to obscure or average discontinuities in the long-axis direction, so that sharp discontinuities on sandy beaches would not usually be detected. The major advantage of trench sampling is the ease of selecting sampling units (a trench is essentially a systematic sample in which all units are chosen), and to some extent the large size of the sample, and the greater depth that can be sampled into the sediment.

A line sample in which all organisms within a certain distance of the line are collected or counted is the rocky intertidal equivalent of the trench sample. Again, horizontal heterogeneity is not examined by this method except in the direction of the line.

In some cases, trenching or line sampling may be applied so that randomly selected parts of each stratum are sampled. This is essentially a linear random sample where the sample units are rectangular, and has the same characteristics of any other linear random sample. The only difference is the tendency to obscure discontinuities in the direction of the long dimension.

Destructive Sampling Devices - Rocky Shores

The unit of sampling on the surface of rocky shores is termed a quadrat, a square or rectangular plot of any size, in which sample removal or in-situ measurements are made. Most destructive sampling on rocky shores can readily be done by using a quadrat frame to delimit the area to be sampled. While the size and shape varies, frames are made so that they bound the entire area to be sampled or have subdivision so that subsampling within the frame is facilitated.

The most common quadrat frame used is a rigid square with flat sides of wood or metal, enclosing a known area such as 0.25 m^2. The sides are graduated at some convenient interval or string or wire is extended from the sides in a grid which subdivides the quadrat. Quadrat frames are further described under photographic methods. The quadrat frame is laid on the surface after the sampling point has been located by methods described elsewhere. A uniform method of placement should be used; the random or systematic point location method locates only a point. The frame can be centered on the point, or one corner can be placed over the point, with the sides of the frame oriented in a standard manner to the transect or stratum boundaries. Sampling usually combines in-situ counts of subsamples of organisms badly damaged by removal, such as barnacles and estimates of plant cover, with total removal of all organisms from the entire quadrat or portions thereof. It is highly recommended that all destructively sampled quadrats be photographed first by the methods described elsewhere, so that the intact appearance is recorded and so that correlations can be established between removal data and non-destructive sampling.

Organisms are removed from the rock surface by careful scraping with strong knife blades or stiff putty knives with the ends bluntly rounded or pointed and the edges ground into a bevel. Paint scraping devices, with short blades bent at right angles tend to damage organisms more than longer blades which can be manipulated easier. Algae with holdfasts inside the quadrat are usually removed intact, but some standard, if arbitrary procedure must be followed for the many organisms of great variety which are overlapped by the frame edges. This is discussed further under photographic sampling. All material removed from the quadrat constitutes the sample, which is most conveniently handled by being placed with its label inside a plastic bag which is securely tied closed. Intertidal biota of rocky shores can be transported alive and stored in plastic bags under refrigeration or even deep frozen until further processing can take place. Handling rigid containers or formalin preservatives not only consumes sampling time on the shore but adds to the hazards of work on slippery rocky surfaces.

Sampling in thick algal mats or mixtures of sessile animals and algae, and sampling multilayered mussle beds requires special devices. The three-dimensional network of spaces present in algal mats and mussle beds usually has an abundant and diverse fauna, many of which are small and motile. If a flat frame is placed on top of such a mat and then scraping is attempted, many of these smaller motile forms will quickly escape. A partial solution is the use of a quadrat frame of metal, with vertical walls or sides and open at top and bottom. Such a frame, either circular or square, can be pressed down tightly against the mat, or forced into mussel beds to minimize the escapement. The frame may also be of wood, or of metal, with a flat flange on the lower side to which a gasket of flexible material such as foam neoprene rubber or foam plastic is attached, to improve the seal when the frame is pressed down on algal mats or barnacle beds. A variant of this type of sampling is to use a frame with such a gasket in conjunction with an agent to kill or immobilize the small animals within. Glynn (1965) used such a frame on the upper algal-barnacle mat on rocky shores, and improved the retention of mobile forms by pouring hot sea water formalin solution into the frame after it was placed. The hot formalin was carried to the site in a thermos bottle, presumably one of steel rather than glass. While hot formalin is effective, the resulting vapors may constitute both a hazard and a significant time delay while the sample is scraped from the rock. Other agents, with less irritating vapor would improve this method; alcohol, acetone, or a mixture of either and dry ice (solid carbon dioxide) are potentially more advantageous, as would be pressurized freon or ethylene chloride, which would freeze or kill organisms by chilling them. Agents such as diethyl ether might be effective by both chilling and narcotization.

Rounded or extremely irregular rock surfaces present special problems also, if they cannot be avoided. Severe parallax problems would result in trying to project a rigid, flat frame boundary onto a rounded surface, and then making decisions as to what to sample beneath it. A flexible quadrat frame can be used to avoid these problems as much as possible. A frame made of a grid or network of rope or other flexible, relatively inelastic material can be carefully applied to curved surfaces to minimize the area distortion, and a satisfactory delimitation of the quadrat area obtained. On extremely irregular surfaces the flexible quadrat frame may be quantitatively superior to a rigid frame, because projection of the rigid frame boundary onto the rounded surface could result in even more distortion of the area boundaries.

Sample Preservation and Storage

Samples from both sedimentary and rocky intertidal areas are usually placed directly into preservatives soon after sieving or collecting. However, in pollution related work where chemical analysis is also required, this may not be possible or desirable. Such samples can be deep frozen until processing, at which time all material intended for chemical analysis can be separated from the rest which should be placed in preservative as soon as it has thawed.

Some advantages can be gained by narcotizing live material first before preservation. If rocky shore samples are transported alive from the shore or conditions permit live sieving of sediment samples, better specimens for length measurements and for taxonomic analysis will result from narcotization. Polychaetes usually constitute a large proportion of the macroinfauna from sedimentary shores and can be abundant in some rocky shore samples. These worms are much easier to work with if they have been narcotized so that they preserve in a relaxed, undistorted state. Place the samples for 30 minutes to one hour in a solution of 7.5 percent magnesium chloride in fresh water, or in 0.15 percent propylene phenoxetol in sea water (McKay and Hartzband, 1970), at a temperature near that of the environment.

Samples are usually preserved in 5 to 10 percent formalin in sea water, and stored in 5 percent formalin or alcohol solutions. The undiluted stock formalin is usually buffered with an excess of borax, calcium carbonate, sodium acetate or hexamine to prevent the final solution from becoming acidic. Gage (1977) has described an improved storage fluid for use after fixation in 4 percent buffered formalin, consisting of 2.5% formalin, 1% propylene phenoxetol, 10% propylene glycol and 86.5% deionized water, buffered at pH 7.5. A conventional solution for storage after fixation in 10% sea water formalin is 5% glycerine in 70% alcohol, either ethanol, methanol or isopropanol. Large bodied species will significantly dilute the original solution in which they are preserved and if formalin is the original preservative, such samples must be transferred to fresh solution for long term storage. The determination of weights from preserved samples is significantly affected by the extraction of soluble materials by both alcohol and formalin solutions. At best, the storage time should be standardized to reduce the variation from this source, but better data will be derived from fresh or frozen samples taken for this purpose, as described elsewhere.

Algae in quantitative samples are well-preserved by formalin-sea water solutions initially, but in time colloids are extracted and the samples will bleach if they are exposed to light. Algae in formalin kept in the dark are satisfactorily preserved until they can be processed. Permananet voucher specimens may be prepared from such formalin preserved algae by floating them in water onto standard herbarium sheets in the usual way.

Measurements Derived from Destructive Samples

The minimum necessary data to be taken from each sample are the species present, their relative abundances, the biomass or an estimate derived from conversion factors using some measurement, and the variance of the abundance of the species. Taxonomic procedures for species identification will not be discussed here.

Biomass may be measured by the wet weight, the dry weight, the ash-free dry weight, the carbon weight, or the caloric content. The units chosen should reflect the importance of the quantity of organic material to the rest of the community, and ideally would be expressed in terms of the calories which would be gained if the organism were eaten. In practice, it is generally impossible to express the biomass with this level of refinement, and either the ash-free dry weight or the dry weight are used. The dry weight is the weight of the tissue remaining after all water has been driven off. The ash-free weight is that weight minus the weight of the inorganic ash which remains after the tissue has been burned. Dry weight may be converted to ash-free dry weight if a conversion factor is calculated, but differences in the organic content with season, reproductive state, or the health of the individual will introduce errors in the conversion. In general, the wet weight of live or dead organisms gives undue emphasis to species which have a high water content but little usable tissue, so wet weight should always be converted to equivalent dry weight or ash-free dry weight. Wet weight should not be used for species which have a variable amount of water retention, such as anemones.

The wet weight is determined after sorting to species by blotting off excess surface water and weighing. Dry weight is determined by drying the species samples separately at about 70°C until successive weighing produces the same weight of the sample. The ash-free weight of the sample is determined by burning the sample in a muffle furnace at 500°C for 12 to 24 hours, weighing the ash, and subtracting the remaining weight of ash from the weight of the dried sample. This presumably represents the organic portion of the sample. Note that organisms which have a large inorganic fraction will be given undue emphasis by wet or dry weights. Shell should be removed if wet or dry weight is to be used, as the shell contributes little to the higher trophic levels.

Samples should be weighed as soon as possible after preservation, as lipids and other organic materials leach into the preservative and would decrease the weight of organics in the sample. In many cases, it is better to estimate the ash-free weight from conversion factors for numbers or measurements to biomass, when the determination of ash-free weight would take too much time and samples would have to remain unweighed for extensive periods. These conversions should be calculated from freshly collected specimens for the best estimate of a suitable conversion factor.

The species list, relative abundances, variances, and biomass of species are sufficient data for most purposes. Various methods using this type of information may be used to evaluate differences between sites or over time at one site. In addition to these, the size-frequency data for the species sampled can be highly informative. Individuals of all species where it is practical to measure size are measured, and the frequency of intervals of size are calculated. The smallest sizes are the newest members in virtually all organisms, so that the size-frequency curves of a species allow estimation of the recruitment to the species at the sampling site. This method is easier to apply to species which have recognizable stages in their life histories, and the frequency of individuals of each stage can be counted. In some species it is possible to observe a seasonal peak in young individuals, and to follow these through time, observing both the mortality of young and the increase in their biomass.

Non-destructive Sampling Methods

Percent Cover on Rocky Shores: Estimation in-situ

A standard method for the estimation of density of macroalgae is the percent of surface area occupied by each species. The algal cover is usually divided into three layers: the canopy, understory, and primary layers. Primary algae are closely associated with the surface, usually encrusting or covering surface area to the exclusion of other species. Understory species extend above the surface but are relatively short, and canopy species are relatively long and overlie the understory and primary layers. Each of these layers is considered separately in evaluating the percent cover of species. The standard method for estimating the percent cover is:

1. A quadrat frame is placed in a selected position according to the sampling design.

2. Within the quadrat frame, the percent cover of the canopy, understory, and primary layers is estimated.

    a. The percent cover of the canopy layer is estimated first. The individual worker must decide whether to include or exclude plants which hang over in the quadrat but have their point of attachment outside the quadrat. Within each of the subdivisions of the quadrat frame, the percent of the area covered by each species of the canopy is estimated by eye. The average of these subarea estimates is the percent cover estimate for that quadrat, for each species in the canopy. An alternative method is to place a transparent plastic sheet over the area, which has 100 or more randomly placed points marked. The alga under each point is noted, and the percent of the total number of points which each species is found under is the estimate of the percent cover for that quadrat. The variance of point-counting is covered elsewhere.

    b. The percent covers of understory species are estimated next. The canopy species can be removed and returned for estimation of biomass in the lab, or they can be shifted aside. Understory species are evaluated for their percent cover in the same manner as canopy species. If the presence of canopy species affects the position of understory species, then the positions of the dots used in the second method above would possibly bias the estimate of percent cover of primary and understory species, if the same random set of points were used in all three layers. If the random-dot method is used, a new set of points should be used for each layer. Rotation and reversal of the plastic sheet provides eight possible sets of random points without generation of new sets of random numbers or making new sheets.

    c. The process is repeated for the primary layer species, using either method for percent cover estimation. The same method should be used for all three layers.

In most studies percent cover estimates are treated as abundance estimates, and the actual number of individuals which contribute to the percent cover in a given area is not estimated. Algal individuals are frequently difficult to define, especially where the species is encrusting and individuals are fused. Biomass is usually estimated in addition to the percent area covered. The estimate of numerical abundance or of percent cover does not give information on the amount of organic material bound in the populations of each species, available to higher trophic levels as food. Algae may be thin and broad, so that a small biomass corresponds to a large percent cover; or may be thick and compact, so that the same biomass is present in a very small area. Percent cover, like numerical abundance, is more suited for comparison of the abundance of one species at several sites than for comparison of the abundance of several species. Biomass, in contrast, is useful for either sort of comparison. If species-specific in-situ surface area, or percent cover to biomass conversions are determined from samples first estimated for cover in the field, then removed for laboratory determination of the weight of each species, the percent cover estimates are convertible to biomass equivalents.

Counts and Measurements in-situ on Rocky Shores

Many species of potential importance are easily measured for size in the field. Size measurements may be readily converted into estimates of biomass if the relationship of linear size and biomass is reasonably consistent. Species of this sort can usually also be counted in the field, since they are large enough to be identified individually. Examples are barnacles (see Connell, 1961), mussels, anemones, starfish, and urchins. The process of counting and measurement in the field is more time-consuming than removal, so that application is limited to situations where there is sufficient time or manpower to gather all the information over one or two low-tide periods.

When a linear measurement is to be used for conversion to biomass, some experimentation with different dimensions will be necessary, in order to select some dimension which is the most highly correlated with the biomass. In many cases, previous work can be referred to for a given species. Destructive sampling to establish the conversion to biomass should always accompany in-situ counts and measurements of size, even when previous work has established conversions in other locations. Populations of the same species may differ in their size-biomass relationship. The conversion factors should also be checked during the study to ensure that the conversion factor has not altered with seasonal changes in the population.

In-situ counts will not be appropriate for all species present at any given site. If diversity is to be used as a major tool for detection of changes in sites, destructive sampling must be used in order to sample the small and mobile species, which cannot be counted or measured in the field. Species for which in-situ counts and measurement are feasible may still be sampled in this manner. The advantage to partial in-situ sampling is that the larger species which may most readily be counted in the field are often rare or slow to reproduce, so that destructive sampling is more damaging to the local population.

It is important to make provisions for comparisons of studies done using different measurement methods. For this reason, non-destructive sampling must always be accompanied by a minimum of destructive sampling to establish conversion factors for percent cover to biomass and numbers and linear size to biomass. Destructive sampling for this purpose should be complete enough so that the conversions can be specified with a known error for the entire range of percent cover, numbers, or sizes found in samples. Even if the biomass need not be known for the particular study using percent cover, in-situ counts or measurements, the study will have lost much of its effectiveness if it cannot be compared to other programs.

Photographic Methods

Introduction

When quantitative non-destructive and periodically repeated sampling of the macroepibiota of rocky shores is required, photogrammetric techniques offer many advantages over direct manual in-situ identification and enumeration. Photography can also be usefully combined with destructive sampling to record features of intact quadrats before removal of organisms. Undisturbed areas reserved for photographic monitoring can be laid out parallel to transects or areas where sampling with removal techniques is used. Photographic methods offer particular advantages of speed to emergency sampling programs initiated with short lead time in cases of catastrophic pollution events. When there is no accompanying removal sampling program which would result in the retention of specimens for identification and mensuration, voucher specimens should be taken from the general site area, but away from the monitoring stations, for taxonomic determination, size and wet, dry and ash free dry weight determinations, for photograph calibration.

Major advantages of photographic methods are:

1. Minimal disturbance of the sampling area in monitoring indicator sites over time.

2. Speed of sampling, resulting in either reduction of sampling time or maximization of the number of quadrats sampled in the time available at low tide, or before some impending event like the beaching of an oil spill.

3. The photographic quadrat or sample may be very large compared with those feasible by manual removal methods.

4. Avoidance of operator errors in data from in-situ counts, resulting from: a. counting difficulties induced by the interference of bad weather, b. inaccuracies resulting from fatigue, distraction of attention by the necessity for avoiding wetting and danger from heavy surf and from the need for hurrying because of time constraints at

low tide, c. data bias from operator decisions when parallax problems occur with small organisms near the edge of counting frames or their subdivisions.

The following types of data for macroepibenthic biota on rocky shores may be obtained by photographic quadrat sampling.

1. The species present.

2. Density (frequency) per quadrat area for each species.

3. Percentage surface area of the substrate covered by algae and sessile animals, including multi-layered algal assemblages.

4. Size-frequency per unit area data for each species with regular morphological features measurable in linear dimensions such as diameter or length.

5. By comparing data from photographic samples from different equivalent places, or different times from the same place, estimates of spatial and temporal variation in the above features.

6. When appropriate conversion factors have been determined for cover or linear dimensions to weight or caloric content, measures and comparisons of biomass weight per unit area and its changes with time may also be estimated from data taken from scaled photographs, without disturbing the sampling site.

The accuracy of a measured frequency of a species is dependent upon the quadrat size used in sampling, the nature of the dispersion pattern of each species and the size of individuals of each species. The ease of both replication of quadrats of a given size and of either varying quadrat size or producing a mosaic coverage of a larger area should allow photographic quadrat sampling to be developed into a superior method for accurately determining the degree of natural spatial variability in frequency related to dispersion patterns.

Using the same equipment, photographic methods are adaptable to many scales of area from quadrats on the order of a square meter for enumeration of large biota to areas of 100 square centimeters for counting recently settled barnacles. Photographic quadrat sampling can also be combined with general photographic recording of landmark features of the site for later relocation without altering the camera equipment.

Disadvantages of the photographic method are:

1. Identification may be a problem with similar species not distinguishable by external gross features if they co-occur at the site.

2. Minute species or those which hide under larger surface forms will be missed in quadrats scaled for larger surface species.

3. Enumeration or percent cover estimates for overlapped species in thick layers is only possible by sequential photographs taken as successive layers are lifted or removed.

4. Initial equipment purchase costs and the operating costs of film purchase and processing may be greater than the costs of manual in-situ identification and enumeration or of removal sampling and the subsequent analysis of preserved samples.

Equipment and Materials Required

Many types and sizes of cameras can meet the few performance requirements for field use in photographic quadrat sampling. Cameras must be easily carried over rough and slippery rocks, readily operated when hand held and withstand damp and salty field conditions without complicated protection and maintanence. Large tripod mounted cameras lose many of the advantages of the method and smaller hand held reflex cameras are generally more suitable. A major requirement is high quality of the final image and hence high quality camera lenses which can operate over a wide range of distances. The great variety of 35 mm single lens reflex cameras, with their "normal" or "normal to close up" lenses are well suited to this work, and subsequent discussion implies their use.

Two cameras mounted on a frame have been used for stereophotographic methods or for simultaneous use of regular color and infra-red color film, for detecting small algae and changes in the condition of algae. A single camera is adequate for all other uses. Both color transparency film and subsequent processing of good quality is essential for the best use of this method. Color produces the contrast and definition required to identify the image of many intertidal species and to distinguish individuals in groups of organisms or from dark rock backgrounds. Color transparencies are readily projected at variable sizes, which facilitates taking data from the film. For some special uses, such as monitoring barnacle populations, color or black and white negative film may be adequate or even advantageous. The main advantage of prints, where negative film is applicable, is that they are permanent records accessible without further equipment. The disadvantages are that ordinary color negative film and printing results in an image somewhat lacking in resolution while black and white prints may lack the visual contrast and definition required for dark, monochromatic species. The time and expense required for print production may offer no cost advantages over transparency material in the 35 mm film size. Prints of adequate tonal range would lend themselves to analysis by automatic image scanning equipment, a procedure not yet applied in this field.

Electronic flash equipment is essential for adequate photography in the rocky intertidal. Even in good daylight, flash is required to eliminate strong shadows and uneven lighting which degrades image definition. Lightweight, easily carried automatic flash equipment with a high light output and capacity for sustained use is preferred. Rocky shore organisms are often dark in color and contrast poorly; an adequate exposure on color transparency film may require a flash duration or intensity two or three times that adequate for conventional photographic subjects, especially when the rocky background is also dark. Consequently, calibration of the flash equipment under field conditions is necessary, even for automatic electronic flash units. Electronic flash units with automatic flash duration or intensity control are preferred to simplify operation in the field where distances will constantly vary between camera and rock surface. Such camera to subject distances will vary from about two meters for large quadrats suitable for counts of starfish, large algae, large sea anemonies and the like, to a half meter or less for small quadrats taken at close distances for enumeration of barnacles and other small organisms. Electronic flash units attached to the camera by an adequate length of cable produce successful exposures in deep tide pools. The flash unit is held to one side, close to the water and at a low angle of incidence to the camera.

Photographic sampling is most effective when done with a gridded sampling frame to scale and define the quadrat space. No records of camera to surface distance or special apparatus for scaling, holding the camera normal to the surface or for corrections for scale and perspective on projection are necessary when frames are used. The frame grid provides an automatic linear scale in two dimensions as well as correcting area for changes in perspective across the field of view. The optical superposition of grid and organism images resolves all parallax problems.

Light quadrat frames can be made of welded rigid, flat aluminum stock, with holes bored along the sides at carefully regulated distances for the grid strings. Frames should be painted a bright color for contrast, such as yellow which will contrast strongly with the background but which will minimize glare on projection. Frames painted white will be so overexposed at exposures required for the organisms that their projected brilliant image will interfere with the vision of the enumerator.

The grid of the frame is produced by tightly stringing the frame with fine, strong white, yellow or orange nylon or polypropylene string, which will stand out sharply from the background. The thickness of the string for a given frame size should be fine enough so that its image cannot obscure the smaller objects of interest. The size of the quadrat frame and its grid spacing varies with the intended use. We have found use for three frame sizes, but a single frame will suffice for most work over a large range of quadrat sizes. For use with a 35 mm camera, frames with side dimensions in a 1:1.5 ratio will most efficiently fill the camera film frame. The consistent use of an orientation mark on the frame edge to denote upward or seaward direction permits the camera to be held with its long frame axis horizontally or vertically as required for positioning for a particular quadrat. Frame sizes proven useful for quadrats varying in area over several orders of magnitude have been one meter square, strung at either 10 or 20 cm intervals, a rectangular frame, 60 cm by 90 cm, with a 10 by 10 cm grid and a small frame with a fine string grid of 3 by 3 cm. The entire frame or a portion is used depending on requirements.

The primary data from photographic quadrats on 35 mm color transparency film is obtained by making counts, identifications and measurements from the projected image of the slides. A good high intensity projector and a high reflectance screen is required for ease in working with large numbers of photographic quadrats. Zoom projection lenses minimize changes in projector to screen distances when adjustment between slides is necessary to produce an easily used image size. Counting and measuring from the projected image requires no special equipment. Hand count recorders permit the operator to record without looking away from the image. White rulers with sharp graduations or dividers are used for measurements. Counting may be facilitated by moving the shadow of an object across the screen as counting proceeds. Counts may be recorded for each grid unit on a numbered diagram of the frame grid. For large quadrats photographed on near-vertical walls significant zonation changes will be present over distances of a few tenths of a meter. Data from different vertical horizons can be easily kept separate on such record diagrams, and horizontal grid rows can be treated separately in data analysis. Photographic transects of rock walls, made by successive quadrats placed at measured levels, are very quick and efficient.

A quantitative photographic method more advanced than that described here is available. Lundalv (1971) and Torlegard and Lundalv (1974) describe a system developed for quantitative studies of the long term changes, including pollution effects, on the epibiota of rock surfaces along the Swedish west coast. A single camera and a reference frame are used to make stereopairs of photographs which are then analyzed for all the types of data mentioned above. Stereoscopy increases image definition and a microstereocomparator available commercially is used for size measurements of great accuracy. As described, the system was intended for underwater use in the shallow subtidal, but is readily adaptable to intertidal use by simplification of the equipment modifications made for underwater use.

When long term monitoring programs or baseline programs with extensive geographical coverage are being planned, evaluations should be made of the cost and relative advantages of conventional sampling and manual in-situ data gathering and of photographic techniques as the major sampling method to be employed on rocky shores.

Photographic Procedures in the Field

The basic techniques for vertical level determination, establishment of transects, stratification, location of sampling points and determination of sample number are unchanged for photographic quadrat sampling. Camera distance is varied to produce adequate size definition of the smallest organisms of interest as well as to enclose the required quadrat area within the camera film frame. Conflict in these requirements may mean that the over-riding requirement of adequate image size will require a given quadrat to be covered by more than one film frame.

The ease with which scale can be changed in photography permits monitoring sites to be initially mapped in detail as a base for later periodic sampling to follow natural or induced ecological change.

For detailed transect coverage, a nested sampling technique may be used by progressively photographing an area covered by a square meter frame for larger, rarer species, and then by moving closer, sucessively smaller sections of the grid, systematically or randomly selected, can be photographed for the enumeration of smaller, abundant species. Repeating this coverage successively at determined tidal heights will quickly produce a detailed sampling of the transect designated.

Photographic coverage is so quick and inclusive of greater area that correct a priori estimation of the minimum number of samples necessary for a given level of precision becomes much less important than when quadrats are enumerated in the field. In practice, oversampling with subsampling later as required becomes a feasible routine.

After the quadrat location is determined, the sampling frame is placed by some convention such as centering it or placing one corner on the location point, with sides oriented to some constant feature such as the transect boundaries, which are usually arranged to be as close to right angles to the waterline as possible. The frame should be held as parallel to the rock face as possible. Commercial rubber cords with hooks or lengths of rubber tubing with a hook made of wire attached to each end can be hooked to the frame edge and to irregularities in the rock face. This will temporarily hold the frame in place and at the right position and permit one operator to both place the frame and to operate the camera. If multiple layers of algae or animals are present, sucessive layer removal and repeated photographs are necessary.

Camera operation will not be described since it is so dependent upon specific design. The use of electronic flash permits smaller lens openings, resulting in greater depth of field and sharper images, as well as simplified operation under adverse conditions. The operator must have previously mastered the operation of the camera and flash equipment. We have successfully obtained photographic quadrat samples from previously determined locations at night by using a bright lantern held close to the quadrat for focusing and thereafter moving the lantern well to the side to avoid glare in the camera lens during exposure.

Record Keeping

In addition to the usual records of location, level and sample designation, records must be made of each photograph. The sequential frame numbering on roll film simplifies record keeping, but rolls must be carefully identified in the field as they are used. It may be sufficient to number the film rolls and record data for each film frame in each roll in the field as they are exposed. Commercial color film processing usually permits individual film rolls to be given an identifying number which is retained with the packaged slides when they are returned. When general orientation photographs of the sample site or region of a transect are included in each roll or on some rolls in a group used at one time, landmarks permit the location of at least larger photographic quadrats to be identified easily. This provides a means for quickly relocating them in the field when resampling the same site.

Each photographic sample may be labelled uniquely, at the time the exposure is made. Pre-prepared paper card labels bearing an identifying sample serial number or letter-number designator written clearly and large enough to be read at the scale to be used can be placed within the field of view, in a corner of the quadrat or on the edge of the sampling frame. If sample numbers cannot be assigned beforehand, or quadrat sizes and hence scales are planned to vary widely, designations can be assigned in the field and marked on stiff cards or pieces of reusable plastic sheet with a wax glass-marking pencil and similarly placed within the frame.

Obtaining Data from Photographic Samples

The processed color transparency is the permanent sample from which the data is derived. The slide, with the image of the frame edges and its grid superimposed upon that of organisms and background, is projected at any size which permits ready identification and measuring, with sufficient image brightness. The grid and frame provide a direct measure of surface area in the quadrat and its subdivisions as well as a linear scale in two dimensions for calibrating any size measurements taken from the projected image.

For photographs taken relatively close to near vertical or near horizontal rock surfaces, where the camera can be held centered on the frame and normal to the surface, corrections in size measurements for differences in near and far dimensions due to perspective, is usually not necessary. The camera cannot always be positioned over or opposite the center of the sample area in some places on vertical walls and on the horizontal, especially when the quadrat is on the order of a meter square. Appreciable perspective effects will be apparent when the slides are projected, and projector manipulation usually results in poor focus of one or the other end of the image. Perspective differences will have no effect on counts made within grid units and no correction is needed for differences in the image size of grid units of equal actual area. When sizes are being measured from the projected image of quadrats with appreciable perspective effect, corrections for successive distances away from camera center may be taken for each row of grid units, from the projected dimensions of the grid sides at those distances.

Edge Effects in Counts

Whether entire photographs are used or areas within them bounded by parts of the frame grid, an edge effect on the frequency per unit area data is produced by operator decisions on the inclusion or exclusion in the count for the unit of individual organisms near the periphery of the unit. This effect is greater for the rectangular 35 mm film frame than from an equal area circular or square frame or portion of the photograph simply because of the differences in the length of the edge relative to the enclosed area, as it is for any rectangular quadrat. Simple solutions to this problem, uniformly applied, reduce its significance greatly.

A decision can be made as to the proportion of an organism which must be within the area to be scored as included. For species with regular shapes, exclusion of all individuals overlapped by a boundary by more than 50% of the dimension used may be an adequate rule. This method requires individual decisions, possibly subjective and liable to bias, and applies poorly to species of indefinite shape and boundary such as some macroalgae.

Other solutions are applicable to both counting and point counting cases where points fall so close to the edge of the projected image of an organism that it cannot be decided if they are inside or outside because of image unsharpness. In point counting, these cases may all be scored as one-half to avoid subjective bias. In frame or grid subdivision counts for density estimates, all cases of actual or unresolved intersection with the frame border or a grid subdivision line can be counted separately and only one-half of these intersect counts later added to the non-intersect count total. For density counting, another method avoiding subjective decisions on the degree of overlap required to be inside the count area is to omit counts of all individuals touched by two adjacent edges or boundaries of the frame or grid units and include all others touched by the other two adjacent boundaries.

Data on Species Presence, Density and Sizes

Species identification, counts and measurements may be taken for all organisms of interest in the entire quadrat area photographed. Preliminary analysis may define the size of a subsample smaller in area than the total quadrat; selection of a subsample of the grid units by systematic or random means will produce the subsample, but subsampling must not confound real vertical differences. Counts of individual grid units should be kept separate and identified horizontally and vertically to permit later analysis of differences due to the tidal gradient when applicable. They may also be used for estimating the contribution of patchiness, on the scale of the quadrat grid units, to the variance of mean density. For quadrats from horizontal surfaces with no appreciable vertical tidal height difference, the frame grid provides a ready means for using rectangular coordinates for random selection of individual grid subunits to count. On more nearly vertical surfaces where there is an appreciable difference in tidal height within the quadrat, the random selection can be confined to units within horizontal rows.

Individuals may be so abundant and crowded in some quadrats that counting proves difficult, even with subsampling. If operator confusion causes difficulty in distinguishing previously uncounted individuals from those already counted, the slide may be projected onto a large piece of white paper and each individual scored with a mark using a crayon of contrasting color. The marks themselves can then be counted, and crossed off as they are used.

Data on Percent Areal Cover

For plants and sessile animals like barnacles, space on rock surfaces may be a limiting ecological resource. Data on the percentage of available substrate space occupied by various species can be used to compare relative density or space dominance within species assemblages as well as to compare spatial or temporal site differences in these features (Littler, 1971, Littler and Murray, 1975). Species occurrence and percent cover data can be very quickly derived from photographic quadrats by the point counting, or intercept, method. In this technique, a regular or random array of superimposed points sample a fraction of the covered area for the presence of the species of interest, with 'hits' being scored. The procedure may be automated by superposition of a point array on the projection screen, a glass plate placed in front of it or over prints, or a point array inked onto a glass plate laid over photographic paper during enlargement. Few quantitative intertidal studies have used point counting on photographs for data on areal fractions (percent cover) of different species to its greatest advantage and none appear to have considered the sources of error and variance produced in application of this useful method. The mathematics of point counting methods has been greatly developed in the field of quantitative microscopy in biology, mineralogy and metalography. A detailed exposition of the statistics and probability involved is given in DeHoff and Rhines (1968).

If a sufficiently large enough number of sample points is used in an array superimposed on the photograph (or part thereof) of a quadrat, the number of sample points falling within the image of individuals of a species will be proportional to the area covered by that species, and the total number of points in the array will be proportional to the total area covered by the array. In practice, the number of hits for each species is expressed as a percentage of the number of points in the array, by simply dividing, to express the fractional area coverage, or percent cover.

Form of the Sampling Point Array

A regular or random array of points or single lines with randomized points along them, are used to sample a fraction of the total quadrat area covered by the images of the species of interest. A grid made by lines joining points in a regular array may be used to effect visual separation of the field while counting. A large projected image may be covered by a smaller point array by systematically moving the projector so that successive parts of the image are sampled. For both regular and random point arrays, the probability of a point falling within the bounds of the image of a given species is equal to the fraction of the total quadrat area occupied by the images of all individuals of that species present in the quadrat. The number of sample points falling within the image of a species is proportional to the area covered by that species, and the total number of points in the array is proportional to the total area covered. The number of hits for a species is divided by the total number of points superimposed on the quadrat image to give the percentage area covered by the species.

The accuracy of percent cover estimates made by point counting methods is determined by the number of observations, or points used, and the spacing of the point array. This spacing, or point density also affects the time efficiency of the method; more points and hence more counting is needed to get a good estimate of the area occupied by smaller species than larger ones. A given grid size, or distance between points in the array used will have different accuracies for large and small species, and for rare and patchily distributed species. The grid spacing should be relatively small with respect to the size of the smaller organisms to be included in the count; it will then be adequate for the larger also. An optimal density of points would be roughly one per individual in the area covered. In regular arrays, a spacing about equal to the average projected image size of the smaller species will be adequate and produce optimal accuracy for the time involved. A systematic array of points will produce less variance due to the size of individuals than a random array, but starting and stopping the point count must be random with respect to any object in the field, when point grids are used.

It is the final projected image size of organisms that is related to the spacing of points in a regular array. For a given size of photographic quadrat, the projection distance and hence the final image size may be standardized and a single grid of a specific spacing (point density) and total size or area used. Any change in the final image size used with a particular grid will result in estimates with a difference in variance, as would its use with photographs of different sized quadrats which require a different final magnification when projected.

The number of points to be counted to give an accurate representation of the smaller species can be adequately determined by trial. Project a typical slide of a photographic quadrat enclosed by the frame size which will be used routinely, and adjust the projector distance or zoom lens until the smallest individual or species of interest can be clearly identified in the projected image. Make an estimate of the average linear dimension of the image of this species, and the length of one of the sides of a unit in the frame grid so that future projection distances for this size of quadrat can be kept the same. Make a grid of 100 points spaced at the average linear dimension determined on a clear glass or plastic plate to be placed flat against the screen or on a piece of heavy white paper to be fastened to a flat support and used as a screen. The total quadrat area will probably project larger than this grid array. Project the quadrat on this grid of points and record all point intercepts, or hits, for the test species, moving the quadrat image across the grid if necessary, to repeatedly sample the quadrat. Using the ratio of hits to total points used, plot the percentage area occupied by that species against the increasing total number of points counted until the curve levels off. This will estimate the number of points needed to be recorded with that point density to get an accurate estimate of cover for the species. This density and point number should be more than adequate for the larger species present.

IV. SAMPLING PROGRAMS FOR SPECIFIC SUBSTRATES

Tidal Flats

Tide or mud flats are broad areas of slight slope which experience little wave energy input, and consequently are composed of fine sediment particles.

Typically, there are variations in the sediment characteristics, often as a result of the drainage pattern of the flat. These are associated with irregularities in the tidal height both parallel and perpendicular to the waterline. Patches of organisms are formed in response to local topography rather than regular series of bands characteristic of more uniform intertidal gradients. In many locations rooted vegetation alters the settlement characteristics locally, producing further heterogeneity on the flat. Rhoads (1974), Kay and Knights (1975) Bloom et al. (1972) and Gray (1974) discuss the nature of animal-sediment relationships in more detail.

These factors contribute to the production of a diverse, patchy environment which is never sampled under the assumption that it is homogeneous. Tide flats are always stratified in some manner, generally reflecting the overall change in tidal height, but also recognizing local irregularities in topography and biotic patchiness.

Stratification of tide flats begins with mapping of the tidal height gradient. Where the slope is readily defined, strata may be bounded by uniform changes in tidal height. The low slope allows small changes in elevation to correspond to relatively large areas, so that it is more convenient to restrict the size of strata by use of smaller changes in elevation than is common in other types of shore. Major swells or basins may be disregarded, excluded from the transect, included as part of the stratification by defining such features as separate strata, or by combining them with other strata of equivalent elevation. Obvious discontinuities, whether physical or biotic, are always recognized. Eelgrass beds, for example, form a separate stratum if continuous along a tidal height. If such beds are discontinuous along the same tidal height, then it may be preferable to separate areas at that height with and without eelgrass. If they are not separated, interpretation should note that eelgrass is not actually found at the average density recorded for the given tidal height. Proportional allocation of sample units follows the identification of appropriate strata.

The large area of tide flats presents some unique problems. If nothing is known about the area, it is difficult to gain information using a preliminary random sample. Positioning of random sample units in a very large area is impractical, and the systematic sample becomes useful as an exploratory sampling method. The systematic sample is more likely to reveal discontinuities in the communities of organisms present at different parts of the same flat, especially when the discontinuities are not related directly to obvious physical changes, or when relatively sharp discontinuities occur over short distances representing nearly unmeasureable changes in elevation. The results of the systematic sample should lead to better stratification of the transects in such cases. If the total number of sample units in the preliminary survey are divided into two samples, then an estimate of the total variability of the transect can be calculated for the estimation of the sample size of the final program. Once the preliminary survey has defined the strata, the final program should be one of stratified random sampling. If the transect area is too large for convenience, transects should be reduced in width to manageable dimensions.

The selection of the transect position is largely arbitrary. Transects should appear at least superficially representative of the entire site, or should be numerous enough to include the obvious variations in the nature of substrate or biota. At least two transects are always necessary for purposes of replication, and in the case of tide flats more are highly desirable.

Temporal variations in physical features are less important on mudflats than sandy beaches, where seasonal changes in wave action can greatly affect the beach slope and sand particle size, or rocky shores, where typically large fluctuations occur in the macroflora and associated or dependent macrofauna. Tideflats do, however, experience considerable seasonal variation in recruitment, and seasonal repetition of sampling is necessary (Woodin, 1974; Reise, 1977; Green and Hobson, 1970).

Sampling Program Development

The steps in the development of a program for sampling of flats are:

1. Identify the number of sites which the sampling program should attempt to examine. Define the absolute minimum number which must be examined.

2. Select a site for the preliminary survey. At this location, take samples of at least five units each, randomly placed within an area 30 m wide, extending from high to low water, with each of a set of cores ranging from about 20 cm^2 to .25 m^2. Table I summarizes core dimensions and sample sizes used in a selection of typical sampling programs on tideflats. All cores should be taken to at least 20 cm in depth, and sectioned into 5 cm or smaller sections to determine depth to which the fauna extend.

3. Determine the optimal sample unit size in terms of the minimum core size which returns most of the species present. For core sizes above this minimum, calculate the relative net precision of each to determine the most efficient coring unit.

4. Assume that at least two transects will be taken at each site, and determine the number of sites which can be adequately sampled. Sacrifice an entire site rather than sample several sites poorly.

5. At each of the sites selected for inclusion in the program, define two or more transects of 30 m width. Separate transects by at least 60 m in order to examine large-scale variability.

6. Survey the beach to establish the profile. Stratify the transects according to the elevation and obvious discontinuities. Note that drainage channels can be excluded from transects unless they are an important feature of the area. Eel grass beds, because of their importance to the estuarine fauna, should be included in the transects as separate strata or in separate transects. Systematic sampling may

be useful to establish where discontinuities are located. Depressions and mounds may be treated separately or included in strata of similar elevation. Strata should be few enough that each stratum is allocated several sample units.

7. Allocate the sample units to strata in proportion to the areas of strata.

8. If possible, avoid sampling strata consecutively from top to bottom or the reverse, to avoid time-dependent errors in sampling procedures. Strata may be sampled in random order.

9. Samples may be sieved in the field or in the lab. Samples should be preserved within 24-36 hours after collection. The smallest sieve mesh should be no larger than 0.5 mm.

10. Sieved and preserved samples are floated, stained, and sorted to species. Species are identified as closely as possible, counted, and weighed for biomass. Dry weight is preferred, but wet weights may be used if suitable conversion factors to dry weight are provided in the report. The size-frequency data should be examined to estimate recruitment.

11. Sediment samples should be taken at the same time for particle size analysis in each stratum. The organic content of sediment may also be useful. This information should accompany the site description. A description of meteorological conditions at the time of sampling should also be supplied.

TABLE 1

SUMMARY OF SAMPLE AND QUADRAT SIZES - TIDEFLAT STUDIES

Name Investigators	Species Studied	Quadrat Size	Sieve Size	Sample Size
Atkinson, R.J., 1974.	*Goneplax rhomboides*	100 m^2 areas	census of burrows	several large areas mapped by divers
Cassie, R.M., and A.D. Michael, 1968.	community	.25 m^2 to 6-10 cm	2.5 mm	5 systematic transects, 40 stations total
Heip, C., 1974.	community	6 cm^2 to ? depth	?	64 cores in 8x8 array at 10 cm intervals to test diversity index
Hibbert, C.J., 1976	12 bivalves	not given	1 cm	4 transects, 200 m apart, sampled at 100m intervals with 2 quadrats per position
McIntyre, A.D. 1964.	meiobenthos primarily	4 cm^2 to 7 cm depth	.5 mm + .076 mm	4-6 cores per station each time, benthic sublittoral area
Myren, R.T., and J.J. Pella, 1977.	*Macoma balthica*	100 cm^2 to 8 cm	3.2 mm + 6.4 mm	One location, 6 transects, 5 strata, 4 sample units per stratum per sampling time.
Stromgren, et al, 1973.	community	.25 m^2 to unspec. depth, or 86 cm^2 to 30 cm	1 mm	

TABLE I, Continued

Name Investigators	Species Studied	Quadrat Size	Sieve Size	Sample Size
Vader, W. 1977.	*Perforatella rubiginosa*	.06 m^2 to 1 cm		Plot positions not given;
Ward, D.V., et al., 1976.	*Uca pugnax*	.52 m^2 to 18 cm		Observations of crabs within defined unit, counts averaged.
Woodin, S.A., 1974.	polychaetes	.05 m^2 to 14 cm	.5mm + 1 mm	A single stratum of one transect 70 m wide, sample 6/yr; also some sampling in other strata
Hughes, R.N. and J.C. Gamble, 1977.	Community	0.25 m^2 to 40 cm	2 mm (5 per site)	All taken at random within 5x5 m square at many locations, tidal heights not determined.
		41 cm^2 to 14 cm	1.6 mm (4 per site)	
		41 cm^2 to 14 cm	0.5 mm (10 per site)	

Sand Beaches

Sandy beaches are typically higher wave energy beaches than are tideflats, and have greater slope covering less area. The slope is usually nearly uniform or changes in slope are easily detectable. The higher energy input reduces heterogeneity parallel to the waterline, so that the tidal height gradient is the dominant one. In most cases, stratification of sandy beaches is a matter of division into equal intervals of elevation, so that strata are a series of bands parallel to the shoreline.

Either line transects or belt transects may be applied to sand beaches. A belt transect is better suited for detection of variation on a scale of several meters, and is preferred on sandy beaches. The width of the transect is typically on the order of 20-40 meters, and transects are usually separated by about twice the width of each transect. This gives an excellent measure of the variation on scales of several meters to hundreds of meters.

A method of sampling which is possible on sand beaches but not generally elsewhere is trenching. This is essentially a line transect where a contiguous series of sample units are excavated in sequence. It is possible because the sand tends to be compact and does not collapse, so that trenches can be quantitative in area and volume. The disadvantages are the same as for any other form of the line transect; variation on a scale of meters is not detected.

High-energy sandy beaches, such as are found on open coasts, are frequently subject to erosion of sand on a seasonal cycle. High wave energy input during winter storms removes sand, while low energy conditions in the summer permit deposition of sand. This strong seasonality in the physical nature of the beach must be reflected in the sampling program by adequate replication through the year.

Sampling Program Development

The steps involved in the development of a sampling program for sandy beaches are:

1. Identify the number of sites which the sampling program should attempt to examine. Define the absolute minimum number which must be examined.
2. Select one site to conduct a preliminary sampling. At this location, take samples of at least five sample units randomly placed over a transect of 30 m width, with each of a range of core sizes from approximately 20 cm^2 to .25 m^2. Table II summarizes core dimensions, sample sizes and number of transects used in a selection of typical sand beach sampling programs. All sample units should be taken to a depth of 20 cm or greater and sectioned into 5 cm or smaller sections to determine the required depth of future cores.

3. From the results of the samples, determine the smallest core size which returns most of the species present. For all core sizes above this minimum, calculate the required sample size and the relative net precision to determine which is the most effective.

4. Determine the number of samples which can be taken when each sample is of the required size for the optimal core, assuming that one sample equals one transect. Assume that at least two transects will be taken at each site, and determine the number of sites which can be adequately sampled. Sacrifice an entire site rather than sample several sites poorly.

5. At each of the selected sites, define two or more transects of 30 m width extending from high to low water, and separated by at least 60 m along the shore, to examine the large-scale variability.

6. Survey the beach to establish a profile. Stratify the beach by uniform intervals of elevation of 1' or 2', unless obvious discontinuities suggest other strata. Determine the area of each stratum.

7. Allocate sample units from the total sample to each of the strata, in proportion to the area of the strata. Place the allocated sample units randomly within the strata, assigning every sample unit a position independently of the others. If possible, avoid sampling strata consecutively from top to bottom, or bottom to top of the beach, to avoid time-dependent errors in sampling methods. Sample strata in random order.

8. Either return the preserved or unpreserved samples to the lab and sieve within 24 hours, or sieve the samples in the field. In either case, unless preliminary sampling indicated otherwise, the smallest sieve size should be 0.5 mm. Larger mesh sieves may be used to remove debris.

9. Sieved samples are floated if necessary, stained, and sorted to species. Species are identified as far as possible, and are counted. The biomass of each species is estimated, preferably by dry weight or ash-free dry weight. Recruitment data is obtained, particularly if life history stages are identifiable. Wet weights can be used if conversions to dry weight are made available.

10. In addition to the biological samples, samples should be taken for sediment particle size and organic content analysis. A complete description of the temperature, humidity, and other meteorological data should be compiled.

TABLE II

SUMMARY OF QUADRAT AND SAMPLE SIZES - SAND BEACH STUDIES

Investigators	Species	Quadrat Size	Sieve	Sample Size
Alheit, J., E. Naylor, 1961	*Eurydice pulchra*	.125 m^2 x 10 cm	2 mm in situ	Transect line sampled at 10 stations, 70 cm intervals. One transect.
Armstrong, J.W. et al., 1976.	miscellaneous	.25 m^2, subs. with 2 15-cm cores, rest to 30 cm depth	1 mm for cores, rest 6 mm in situ	5 beaches, 7 transects per beach. Each transect duplicate units at 0,3,6' above MLLW. Also special studies of different design.
Baxter, R.E. 1971.	clams	minimum 20 linear feet by 8" by 1 ft deep.	range to 1mm	Several beaches, one transect per beach. Trench 8" wide, 1 ft deep, at least ½ of each 1 vert. ft. interval dug as quadrat.
Birkeland, C. et al 1976.	miscellaneous	.1 m^2 if emmersed .025 m^2 if immersed apparently to 2"	.5 mm	One beach, one transect, three strata, 8 sample units per stratum in random array.
Dauer, D.M., J.L. Simon, 1973.	miscellaneous	combined 5 cores of .008 m^2 area per sample unit	.5 mm	One beach, one transect, four stations per transect. Five cores=one sample unit, five units per station.
Holland, A.F., and T.T. Polgar, 1976.	miscellaneous	.25 m^2 x 25 cm		One sandbar, four stations along center. One unit per station. Replicated six times through 1.5 yr.
Hubbard, J.D., 1971.	miscellaneous	.25 m^2 x 30 cm		Several beaches, one transect per beach, contiguous series quadrats each transect.

TABLE II, Continued

Investigators	Species	Quadrat Size	Sieve	Sample Size
Hummon, W.D. 1974.	miscellaneous	10 cm^2 cores horizontally at each depth interval, unstated distance in.	flotation	Two beaches, one transect per beach, $2\sqrt{d}$ stations per transect (d=width beach), series of depths per station, 3 cores horizontally at each depth.
Kaiser, R.J. 1976.	*Siliqua patula*	.1 m^2 x 31 cm subunits within 20 m squares	"fine"	Unstated beaches, transect arbitrarily within clam popln boundries, 3 sample units within each plot. Proposed plan only.
Mattheisen, G.C. 1960.	*Mya arenaria*	.0625 m^2 x 5 cm	1x1.5 mm in-situ	One beach, eight transects at 10 m distance. Sample units taken every 10 m along each transect.
Nybakken, J.W. 1971	miscellaneous	.25 m^2 x ?	?	Unstated beaches, 5 transects per beach, contiguous series of quadrats.
Southward, A.J. 1965.	miscellaneous	recommends .1-.25 m^2 to 20 cm	.2-.5 mm	Recommendations only, several transects per beach
Zimmerman, S.T., and T.R. Merrel, 1976	miscellaneous	.01 m^2 x 10-20 cm	?	Apparently 1-3 transects per beach, depending on length and variability.

Rocky Intertidal Shores

Introduction

Rocky shores typically have greater relief than sandy beaches or mudflats. At a given site, local topography may range from shallowly sloping benches to vertical rock faces. Wave exposure may range from very protected to highly exposed on opposite surfaces of a single outcrop. Both of these gradients are of importance in determining the nature of the community present. Extremely exposed areas often have reduced diversity, with relatively few species adapted to severe wave shock. Very protected areas may be diverse, or may be dominated by virtual monocultures of an algal species, while areas of intermediate expsoure can present the most diverse communities. The slope of the surface affects the degree of wave shock, since benches tend to dissipate wave energy more than vertical faces. The slope of the surface also affects the rate at which water drains an area, which in turn controls both dessication and the availability of food to filterers. Each combination of wave exposure and slope can potentially support a somewhat different community, so that replication of transects must reflect these physical factors. In the rocky intertidal, replicate transects must share the same approximate degree of exposure and slope.

Identification of the physical characteristics of the site in the rocky intertidal is obviously the most important part of the planning of sampling in the field. Conditions of wave exposure are difficult to measure (Field, 1968; Jones and Demetropoulous, 1968), but in most cases a subjective comparison of locations is sufficient and precise quantification is not necessary for site selection. The slope of a transect may be measured by the usual means described in the section on transect surveying. Minor differences are not likely to be crucial, so that relatively crude comparisons of slope will suffice.

Two additional features which potentially affect the community composition are the type of rock substrate, and the minor relief of the rock surface. The hardness of the substrate will affect the success of boring and attachment, while extensive sculpturing of the surface on a small scale provides small crevices which can support many refuge or opportunistic species, a diverse crevice fauna, or in the case of high-exposure areas, provide refugia for species which could not maintain themselves on open surfaces. Replication of transects should attempt to recognize these factors in addition to the previous two. Dahl (1973) presents a method for the approximation of the actual surface area of a convoluted rocky surface, which can be used to identify the degree of relief of a surface. The method compares the type of relief sculpturing to any of several theoretical surfaces and calculates an index expressing the increase in surface area by convolution over the area of a similarly bounded planar surface. The method can also be used to describe the larger-scale relief of an entire site, should quantitative comparison of the physical (topographical) variability be desirable. Harlin and Lindbergh (1977) discuss the importance of small-scale surface relief to recruitment of macro-algae.

A given transect should extend from high to low water extremes. Within the transect, changes in the slope of the surface should be represented in the stratification, so that strata do not include two areas of greatly different slope. If a given transect crosses areas differing greatly in wave exposure, then the strata should separate areas of different exposure.

The order of factors controlling the stratification of a transect is: obvious zonation in the community, tidal elevation, slope, exposure to waves and sun, substrate composition and relief. If possible, however, the exposure and slope of the surface should be grounds for establishing separate transects rather than separate strata within single transects. Two transects should not be considered as completely replicating each other unless they are in areas which are subject to about the same physical conditions. Since replication within a site is important, a very heterogeneous sampling location may require that several transects be sampled in order to provide replication of sampling under given conditions. If only a limited representation of the area is possible for practical reasons, sampling should be concentrated on the most common conditions at that site. A site which can generally be categorized as protected, for example, might not be sampled in a few areas which are exposed to severe wave shock. Outcrops and their vertical faces may be disregarded in areas where the major feature is a shallowly sloping bench.

In most cases within a given sampling location, the spatial extent of areas which are subject to the same conditions will be small. Transects on rocky shores are not as wide as those on tideflats or sand beaches because of greater physical heterogeneity. The width of a transect controls the spatial scale at which variations in the biota are detected. Rocky shores are commonly highly variable over shorter distances than unconsolidated shores, so measurement over smaller distances is appropriate. In some cases, the available area for a given transect may be so small that the transect becomes a single line of sample units. The positioning of sample units in this case is the same as described for larger-area transects. Within the smaller areas, the preliminary survey will allow assessment of the required sample size for estimation of the mean densities and associated variances of the biota. Table III summarizes some information from a selection of rocky shore studies which may aid in planning preliminary surveys.

In planning sampling programs for rocky shores, a decision must be made whether the sampling plan will attempt to sample the total biota present in all microhabitats. Because of the high degree of spatial heterogeneity and resulting diversity of distinctive habitats occupied by specialized species assemblages, an effort to sample all of these conditions will require very extensive committments of time and resources of the sampling program.

Rocky shores have distinctive cryptic faunas occupying both physically and biologically created spaces such as holes, crevices, undersides of boulders and ledges, dense algal mats, algal holdfasts, and spaces below and between sheet or mat-forming animals such as mussels and barnacles. Only that portion of the cryptic fauna associated with larger surface forms will be sampled with the surface macrobenthos. In some cases, like the faunal assemblages of kelp holdfasts (e.g. Jones, 1972, 1973), the quantification of these assemblages has been used to detect pollution effects, by special studies of them alone.

For other rocky shore cryptic spaces, no methods are known for routinely and quantitatively sampling the fauna of borers, nestlers and other occupants of crevices and holes in rock. These habitat spaces themselves are extremely erratic in their horizontal distribution at any tidal height, but both borers and more passive crevice seekers show distinctive distributions in the vertical tidal gradient (Kensler, 1967). Most of this fauna is specific for cryptic spaces, and while some are found both in rock cracks and the spaces enclosed by larger surface organisms, none are found both free on surfaces and in crevices.

A sampling program which mixes data from samples of biota from open rock surfaces and that from crevices will reduce the definition possible for either habitat, and the resulting data will contain unnecessary variation. If cryptic spaces are to be sampled, the variety existing in the study areas should be divided into defined categories, each of which should be sampled separately in a plan stratified with respect to tidal height. The erratic distribution of those spaces not resulting from other larger organisms will probably preclude sampling in transects, and quantification of both the amount of cryptic habitat available and its biota will be difficult. The work of Dahl (1973) will aid in planning such work, as will the example of Stearn, Scoffin and Martindale (1977) who applied Dahl's considerations to determining the actual area of a fringing reef, on several scales of relief.

It must be recognized that sampling of the cryptic fauna requires the destruction of the habitat being sampled, temporarily in the case of the biologically created spaces, permanently in the case of spaces within the rock itself. The cryptofauna are obviously unsuited for monitoring studies since undisturbed sampling is impossible. This group of species raises the question: how much of the total community or species assemblage of a place must be sampled to characterize the community in contrast to exhaustively cataloging it, especially for the purposes of early detection of pollution effects and their regulation. The precision gained from restricting the work to a dominant and clearly characterized segment, in this case the biota of open surfaces, appears to be preferred to the detail resulting from inclusion of additional microhabitats at the sacrifice of time and resources which could be used or replication at some scale between the sample unit and the site.

Another distinctive minor habitat of rocky shores is comprised of tidepools of various types. On all rocky shores some depressions are found which hold variable amounts of water at low tide. They are the consequences of highly variable geological and physical processes and only those directly produced by the actions of organisms, like the pools resulting from the shallow boring actions of sea urchins, show any regularity of horizontal or vertical distribution. Because of their highly irregular and unpredictable occurrence, few species are exclusively tidepool specialists, unlike the fauna of crevices, and found only in this microhabitat. Almost all of the biota of tide pools located at any tidal height, save upper spray zone pools, are species characteristic of the level at which they are found, mixed with species from lower levels, in effect displaced upward by continuous submergence and which are tolerant of the other physical and biological characteristics of pools. Tidepool salinities and temperatures, as well as oxygen content may differ markedly from the adjacent open sea and the conditions in the rest of the rocky shore.

If a sampling protocol of any type permits sampling points to fall without distinction within pools and on draining surfaces, the resulting data will again contain erratic variation in number and level of organisms. This may reduce the capability of resolving differences otherwise detectable between sites and levels. Tide pools can be sampled quantitatively with sufficient resources, and because of the sharp definition of area possible may make good monitoring points for repetitive non-destructive sampling.

Perhaps the simplest solution to the presence of pools in areas where transects are planned is to limit the major program to quantitative sampling on open, draining surfaces with a constant orientation to the open sea and incoming waves. The resulting samples will be restricted to a more uniform assemblage of open surface forms and their cryptic associates, with good fidelity to tidal height and thus amenable for use in spatial and temporal comparisons of variations of presence and abundance and their possible relation to disturbance. Pools may be largely avoided by an arbitrary rule of excluding surface samples from all pools of standing water deeper than 2 cm and having a surface area approximately that of the sampling quadrat in use.

If tide pools constitute important habitats for the study or comprise a significant amount of the surface area of sites under study, and time and effort resources permit, a separate sampling program for pools alone may be justified by the goals of the program. Replication of sampling within pools of any significant depth should distinguish between the side walls and the bottom as well as between different depths within the same pool. Replication between pools requires that pool depth, surface area and volume be matched or controlled in some way, as well as the tidal height of the pools to be compared. Rocky shore irregularities make close replication at a given tidal height of pools with the same physical characteristics difficult.

A serious effort to include in rocky shore surveys those fishes which do not depart with the tide will require that tidepools, including those at the base of boulders, be sampled. Because these small fishes are highly mobile, cryptically colored and formed and seek shelter, such an effort will require that poisons, anaesthetics or total emptying of water be used. In either case of the use of chemicals, a change is made on the biota of the pools. Total removal of water by bailing or pumping, followed by exhaustive searching of all crevices and algae in the pool is necessary if the sampling is to be non-destructive. Destructive quantification with poisons like rotenone or anaesthetics like MS-222 (tricane methanesulfonate) and Benzocaine (ethyl-p-aminobenzoate) and draining and searching is possible. Any tide pool sampling should be accompanied by data collection on pool vertical height, surface area, depth volume, length of actual margin and some measure of physical and biological cover.

Sampling Program Development

The steps in the development of a sampling program for the rocky intertidal are:

1. Identify the minimum and desired sites to be sampled.

2. Select a typical site for the preliminary survey.

3. Identify, within this site, the gradient from exposed to protected areas, and select a vertical face and a shelf area. If either gradient is minor it may be ignored. The preliminary sampling should examine a position which is intermediate between the extremes of exposure, unless it is possible to sample both extremes. The major slope of the area should be used as the preliminary transect location. If both vertical and level surfaces are important, both should be sampled. At each selected part of the sample location, a transect should be defined from the upper to the lowest extension of the rock surface which has about the same slope and exposure. In some cases a vertical face may not extend the full range, or a shelf not extend to the high water line. Each transect should be as wide as possible for the area with the desired characteristics, to 20 m. Line transects should be avoided.

4. At each transect, at least five sample units (quadrats) should be taken at random positions in the transect, with each of a range of sizes from approximately 400 cm^2 to .25 m^2. Table III will aid in quadrat size selection. If there is a great difference between the upper and lower intertidal, it may be more effective to separately sample each half (to stratify) for a better estimate of the variation of the site. Stratification may also be used for each obvious discontinuity, provided that each stratum has at least two sample units.

5. Each sample unit (quadrat) is sampled as follows: a) The percent cover of macroalgae for each layer (canopy, understory, primary layers) is estimated by either the point-intercept method or the grid method. b) All macroalgae are removed and retained for biomass determination. c) Large invertebrate fauna are removed and retained for biomass and counting. d) The surface is scraped clear of small and attached fauna for later counting and biomass. In general, subsampling should not be done at this stage, since this information will be used to determine the future sample unit size and sample size.

6. Determine the optimal sample unit size for the number of species recovered, for the various communities sampled in different transects. For the quadrats above this minimum size, determine the relative net precision for each sample unit size, and select the sample unit which is most efficient. Since this may vary between transects, use a conservative decision rule, selecting the sample unit which is most

suitable in all parts of the sample location. Calculate the required sample size for this quadrat. This will also differ between transects, so choose the largest sample size determined and use this sample size in all transects.

7. Based on the required sample size, determine the number of transects which the program can support and determine the number of sites which are to be sampled with at least two transects; more transects should be allowed per site if there are significant differences in exposure or slope in parts of a site.

8. At each site, select at least two transects which incorporate the range of exposure and slope in the site. Each characteristic area should be replicated if possible (two exposed transects, two protected, two vertical faces, or two shelf transects). The transects should extend as far up and down as possible, and be as wide as possible under the same set of conditions.

9. Survey the transects. Strata are defined on the basis of height or overriding biotic discontinuities. Allocate sample units proportionally to the strata, and position them randomly in the strata. Each sample unit is sampled as in the preliminary sampling. If a large unit has been selected and time becomes limiting, subsamples may be taken at random within the larger unit. In general, macroalgae and larger fauna should be sampled in the entire frame, and if necessary small fauna sampled in a subsample of at least 20% of each quadrat.

10. Samples are preserved in the field or returned to the lab for sorting and preservation. Samples are floated, stained, and sorted to species. Size fractionation may be obtained by sieving. The biomass of each species is determined. Recruitment is estimated if possible.

11. Within areas where boring and crevice fauna seem important, after the standard quadrats are scraped, one or two may be chiseled out to examine boring fauna. Crevices may be selected at random for qualitative sampling. These are pried apart and the fauna floated and sieved free of the usual accumulation of debris.

12. Meteorological conditions during sampling are provided in the site description.

TABLE III

SUMMARY OF INFORMATION ON ROCKY SHORE STUDIES

NAME INVESTIGATOR	SPECIES	QUADRAT SIZE	SAMPLE DESIGN
Barr, L. 1971	Strongylocentrotus species	diver sampling, .25 m^2 quadrats	Five sites selected around an explosion center, plus one control. 20 selected plots by random throws, using weight to select position without diver bias. Sites selected with bias for density or algal cover.
Birkeland, C., et alia, 1976	miscellaneous	.125 m^2 x 6-8 cm	Intertidal reef shelf. Transect perpendicular to shore, separated into obvious zones of communities. 4 quadrats selected in random method from 2-d field. Quadrats chiseled to 608 cm.
Birkeland, C., et alia, 1976	Laurencia papillosa,	.06 m^2	Biomass variation of one species - a narrow zone on above shelf. 36 (apparently) quadrats in random 2-d array in zone, rejected if greater than 50% other species or in pool.
Birkeland, C., et alia, 1976.	Littorina, Tetraclita Chthamalus, Abietinaria	.125 m^2	Andesite rock bench sampled in four zones of species, 4 sample units per zone in random array.
Boalch, G.T., N.A. Holme, N.A. Jephson, JMC Sidwell, 1974	miscellaneous	1 m^2, subs with .01 m^2 or with .25 m^2	Slate reef, three radiating transects from center of reef, one down reef center, two to low water line. % algal cover by length of tape intercepted by a species along a line.

TABLE III, Continued

NAME INVESTIGATOR	SPECIES	QUADRAT SIZE	SAMPLE DESIGN
Chan, G. 1973	miscellaneous	1 m^2 subs. with .01 m^2	Apparently one transect with many strata. Each stratum a perpendicular line transect 10 m long, divided into contiguous 1 m^2 quadrats, either subs, or total sampled.
Chow, V., 1976.	*Littorina scutulata*	15 x 15 cm	Apparently two transects, one in a sheltered area and one in an exposed area. Line or maybe belt transects separated into .1526 m strata, 5 quadrats per stratum.
Clark, R.C. et al, 1973.	Miscellaneous	30 x 30 cm, subs. 10 x 10 for limpets+barnacles, 10 x 30 for mussels	15 sites sampled once per month for 3 months, then once at 6 and at 9 months. Each site sampled at 1 quadrat position per each "life zone" evident in perpendicular transect. Photographed and counted. Species list within 5 m of each quadrat.
Connell, J.H., 1961	barnacles	.1 m^2	Single location, 8 positions chosen as the first 8 with more than 50/.1 m square. Photographed for time progression.
Feare, C.J., 1970.	*Nucella lapillus*	25 ft x 3 in for census, .25 m^2 for growth studies	One location, a sloping shelf. Four transects 375 ft long and 10 ft apart, perp. to shoreline, divided into 15 25' sections as sample units. Growth studies used 4 .25m^2 sample units in each of seven (undescribed) strata. Sample units of arbitrary size when pop. was aggregated in winter.

TABLE III, Continued

NAME INVESTIGATOR	SPECIES	QUADRAT SIZE	SAMPLE DESIGN
Frank, P.W., 1975	*Tegula funebralis*	100,900 or 2500 cm^2	Selected sites along coast. At each site a grid set up, and random quadrats with size dependent on density collected until 500 snails were obtained.
Kennedy, V.S., 1976.	mussels, 3 spp	.1 m^2	A contiguous series of quadrats through the intertidal on a vertical rock face, all mussels taken.
Lees, D.C., R.J. Rosenthal, 1975.	Suggests sizes and frequencies of sampling for different areas and species, depending also on the proposed information to be obtained.		
Littler, M.M., 1971	Coralline algae	.07 m^2	The area was divided into strata of equal area by unspecified means and a variable number of throws used to establish random sample unit positions. Strata about 20x140 m.
Littler, M.M. and S.N. Murray, 1975.	macroorganisms	probably .07 m^2	Five radiating transects from outfall plus two control areas 50 and 100 m away. Controls randomly placed perpendicular to shoreline. Two seasons used, combined in analysis. % cover by point-intercept method.
Menge, B., 1972.	*Leptasterias* and prey species - small larger large	10 m^2 100 cm^2 1600-3600 cm^2 1 m^2	Unrecorded number of sites, 2-4 transects per site. A single set of random marks along a line used for all transects for quadrat positions.

TABLE III, Continued

NAME INVESTIGATOR	SPECIES	QUADRAT SIZE	SAMPLE DESIGN
Menge, J.L., 1974.	*Acathina punctulata* and prey - small large/rate	24 m^2 subs .01-.16m^2 .36-1m^2	A permanent quadrat marked at one site and subsampled, until 100 individuals per species were collected.
Moore, P.G., 1972.	holdfast fauna	one holdfast	15 sites selected in uniform intervals along coast, 5 holdfasts selected at each as units of the sample of each site. Holdfast choice by a sort of nearest-neighbor selection process from the first suitable holdfast.
Nybakken, J.W., 1971.	miscellaneous	.25 m^2	A grid surveyed at each site, .25 m^2 sampled at each station randomly located on the grid.
O'Clair, C.E. and K.K. Chew, 1971.	macrofauna small	.25 m^2 subs. 5 cm	Unrecorded number of transects divided into contiguous quadrats and strata on the basis of substrate and algal cover. A single plot at each stratum chosen subjectively.
Paine, R.T., 1971.	*Tegula funebralis*	.026 m^2 or .1 m^2	Single site chosen and separated into sloping bench and non-bench strata. 22 quadrats were placed in entire area, not stated if prop. allocation used. Random placement.
Reimer, A.A. 1976.	*Tetraclita stalactifera*	.125 m^2	A single site was sampled three times with four random quadrats along the center of the zone of the study population.

TABLE III, Continued

NAME INVESTIGATOR	SPECIES	QUADRAT SIZE	SAMPLE DESIGN
Russell, G. 1973.	miscellaneous	.25 m^2	One location. Five quadrats were placed at 1 vertical ft intervals and % cover and faunal density recorded.
Sammarco, P.W., Levinton, and Ogden, 1974.	Diadema antillarium	.25 m^2	Five patches of reef previously cleared of Diadema were sampled by 6 quadrats each, laid along a compass direction at uniform intervals.
Sutherland, J.P., 1970.	Acmaea scabra	400 cm^2	Two zones identified. 21 quadrats split 18 and 3 in the zones, every 2 months censused for presence of marked individuals.
Underwood, A.J., 1976.	4 species gastropods	.25 m^2	16 quadrats permanently marked at one study location, censused for target sp monthly for one year.
Zimmerman, S.T., T.R. Merrel, 1976	miscellaneous	1/16 m^2	1-3 transects perpendicular to shore, depending on fetch. 15-50 m, 2-3 lines; 75-200 m, 1 line. Quadrats at regular intervals along transects. Boulders by random selection.

Gravel and Cobble Shores

Introduction

Open coast beaches, which are unconsolidated, but in which the particle size ranges from fine gravel to boulders, are typically high-energy, harsh locations characterized by relatively low diversity. The slope is generally steeper than on a sandy beach, with distinct changes in slope reflecting summer and winter wave conditions. These features are more pronounced than on sand beaches.

Few studies have been conducted on open coast gravel or cobble beaches, but they may be thought of as the extreme case of high-energy sedimentary shores, and do not present problems fundamentally different from those which may examined in sandy beach environments. One such study is that of Paul and Feder (1973), who sampled two gravel beaches and one mudflat in examining the distribution of _Protothaca staminea_. The sampling design was the same as that used for the mudflat, using a single transect per beach, and the number of sample units was a function of the width of the beach.

Within embayments and estuaries, cobble beaches built at times of extreme river floods or past episodes of glaciation may now be very stable; these share features with both rocky and sandy shores. The stable surface rocks afford attachment places for hard substrate species; their interstices and undersurfaces have a fauna analagous to the crevice fauna of open shore rocks, and finally there is an infauna within the mixed sediment below the surface. The algae and animals of the surface, both sessile and sedentary forms, must be sampled by the quadrat methods described for the open coast rocky shore. The effect of increased surface area produced by the cobble relief may appreciably affect percent cover estimates as well as density. Because of the great variability possible in the size of the rock particles present, no one method is applicable for these shores. It may be necessary to use sample units of quite different area for epibiota and infauna estimation.

The basic sampling design on gravel to cobble beaches is the same as for sandy beaches, in that transects of moderate width are defined, strata selected on the basis of tidal elevation (and in this case, changes of slope), and random sampling conducted within the strata. The number of transects will usually be the same or slightly fewer, since the greater energy input tends to reduce the heterogeneity of the substrate.

Sampling Program Development

The steps in the development of a sampling program for sedimentary substrates of gravel and larger size are:

1. The sites to be sampled, including an absolute minimum number, are identified.

2. Select one site of each sediment type (gravel or cobble) as the preliminary sampling site. At these locations, take samples of at least 5 sample units randomly placed within a representative transect about 30 m wide, with each of a range of sample unit sizes from about 400 cm^2 to .25 m^2 in area.

3. Each sample unit is taken by: a) random placement of the unit b) removal of algal cover, if any, from the surface of the upper cobbles c) excavation of the sample unit to a depth of at least 20 cm, and preferably 30 cm. Flotation may be more useful than sieving in separating out organisms.

4. Determine the best sample unit size for retention of the majority of the species. For the sizes above this minimum, calculate the relative net precision and the associated minimum sample size, and select the optimal sample unit.

5. Determine the number of sites which may be sampled, assuming that each site has two or more transects. Delete entire sites rather than sample some site insufficiently.

6. At each selected site, define the transects using a width of about 30 m. Transects should be separated by at least 60 m to estimate large scale variability. Survey the transects, and stratify the transects according to changes in the slope of the beach, or to uniform changes in the elevation of about 2'(60 cm). Determine the area of each stratum.

7. Allocate sample units proportionally to the size of the strata. Place sample units randomly within each stratum, and if possible sample strata in random order rather than consecutively to avoid time dependent errors in sampling. The sample units are taken in the manner described above. Note that larger sediments will require that a series of sieves be used to avoid damaging the finer sieves.

8. Samples are either separated and preserved in the field or returned to the lab; normally field separation and preservation would be done. Samples are floated free of debris, stained, and sorted to species. Species are identified as far as possible and counted, and wet or dry weight is measured. Sizes of individuals or numbers of life history stages are noted if possible to evaluate recruitment.

9. Samples are taken at the same time for analysis of sediment particle distributions, organic detritus content. A description of atmospheric conditions is compiled for the sampling period.

V. DATA ANALYSIS

Introduction

The design of any sampling program, including sample type, size, arrangement, etc. depends upon identification of the question to be investigated and the statistical data analysis technique to be used. Studies of specific pollution effects such as the effect of a pollutant issuing from an intertidal point source can be planned around specific questions, such as the nature and extent of the effect on intertidal community characteristics at distances from the source or determining the distance and dilution at which no community effect is detected. The appropriate data analysis methods are ones testing specific hypotheses, and the sampling program is then designed to produce data meeting the requirements of the specific analysis to be used.

Such a study design will differ from a baseline or monitoring study intended to detect any changes originating from any of a variety of potential perturbations of unknown probability. In this case the goal of the study becomes a general characterization of important and readily measured features of the intertidal community. Requirements of design differs and data analysis becomes a problem of classification and characterization rather than the statistical testing of specific hypotheses.

Every study will have its own design and data analysis requirements and no plan can be given which will meet all contingencies. As a first step in any program development, the problem or question to be investigated should be clearly stated in as specific a form as the case permits, and analysis methods appropriate to the question selected before the final plan is formulated. The details of the sampling design can then be specified to meet the requirements of the problem and the intended method of data analysis. This will also provide a rational basis for any decisions on limiting the sampling to conform to realities of time, budget or other limitations. It is probable that, as a consequence of limits to the understanding of the potential effects of perturbations of all types on the intertidal community, most studies will include some requirements for data to be used in testing specific hypotheses as well as in analyses like clustering techniques which test no particular hypothesis.

The following sections will briefly review different types of data analysis, largely from the viewpoint of types of questions or general ecological information each is most suited for in pollution related intertidal investigations. References are supplied which permit the techniques to be adapted to specific programs. Once the problem has been identified, this section will illustrate how various techniques of data analysis can aid in its resolution. The final sampling design should reflect the specific statistical requirements of the techniques chosen for the study.

Standard Ecological Information

The minimum information which should result from any study of the communities present in various locations is a list of species present in each sampling location, the absolute abundance of each species in both numbers and biomass, and the variability of the abundance over space and through time. These data are generally adequate to compare the similarity of the communities present at each location, and to evaluate changes in a community at one location through time.

Comparisons are also possible on the single-species level, for differences in the abundance of selected dominant species through time or in different locations. The computation and comparison of means and variances are discussed in great detail in many standard statistical texts, and will not be covered here; one such suitable text is Snedecor and Cochran (1967). Elliott (1971) provides a very useful description of the procedures used in the analysis of benthic samples for streams, which are often exactly the same procedures as would be applied to samples from the intertidal. Simple comparisons of the mean density of species in different locations or at different times will usually be quite straightforward; the sole caution would be to note that most count data will have a roughly negative binomial or other clumped distribution, and therefore transformation of the data will be necessary in order to use parametric statistics. Elliott notes that samples over 30 units in size may assume normal distributions, and provides suggestions for transformations for small samples with contagious distributions. Both Elliott (1971) and McIntyre (1971) suggest the transformation $\log_{10}(x+1)$, for contagiously distributed data.

In addition to the basic data, several other types of information can be very useful; among these are the size-frequency or its equivalent, the age-class frequency distribution. This is used to evaluate the recruitment of young to the population and in some cases the recruitment success of past years, when the absence of an age class suggests that a past year has been stressed (assuming that year classes are separable, see Yong and Skillman, 1975; Hancock, 1965 and Hancock and Urquart, 1965). When the frequency distribution can be expressed as discrete intervals, a chi-squared test may be used to compare the size-frequency distributions of two locations, or one location at two instances.

The health of individuals may be of use in determining whether pollution or other disturbances have affected populations. Some measures of health are the reproductive capacity as measured by gonad weight or content, the weight per individual, or the regression of weight on some independent measure of size, such as length.

The basic data listed above may be summarized by various statistics which may be termed community composition parameters. These measure characteristics of the community as a whole. The most commonly used parameters are diversity measures and measures of evenness or its complement, dominance. These are discussed in more detail in the following section. In general, diversity indices are parameters which express the composition of the community, in terms of the species present, their relative abundances within the site, and in some cases, the overall abundance of the entire assemblage at a site. Examples of the use of diversity indices to describe marine communities are Fager (1972), Johnson (1970) and Sanders (1968). Changes in the diversity of a community through time may be assumed to indicate that the site is undergoing some fundamental change from a stable equilibrium of species, or is recovering from a disturbance and is returning to the original or to a different stable point. The direction of the change may be toward greater or lesser diversity; studies have shown that diversity may increase or decrease when a site is disturbed (Zimmerman and Livingston, 1976; Grimes and Mountain, 1971). It is the change in diversity rather than the direction of change which may serve as an indicator of disturbance effects.

Evenness and dominance indices, respectively, measure the degree to which species are present in equal or in greatly disparate numerical abundances, versus the case when one or a few species dominate. Like diversity indices, the evenness or dominance of a site may increase or decrease following a disturbance, depending on which species are susceptible and which are resistant. It is again the change which serves to indicate that the disturbance has had an effect.

When two sites are compared by any of the community composition parameters, the comparison is almost meaningless if the sites are not essentially replicates in their potential species compositions (Jumars, 1974), at least for the purposes of the type of studies discussed here. A shift from two replicate sites being essentially identical in diversity, to one site having a greatly different diversity, is a strong indication of disturbance at the site showing the change in diversity. The same magnitude of change when the sites differ greatly in environmental characteristics provides almost no information on whether the change was due to normal or unusual causes. Selection of control sites on the basis of similarity of environmental factors and superficial similarity of species composition will often be adequate. However, innumerable subtle environmental factors result in stable natural differences in community composition and zonation on rocky shores; many examples are given in Lewis, (1964). Control sites in this habitat type must be selected with care, and preferably on the basis of preliminary surveys. More detailed comparisons may be made using preliminary samples and computing any of several possible measures of diversity, discussed in a later section.

Population and community statistics are greatly affected by various scales of non-random dispersion. Fager (1972) determined that Sanders (1968) rarefaction method for the determination of benthic diversity, for example, was biased when aggregation properties differed between species. Numerous papers have dealt with the effects of aggregation on estimates of mean density; these are summarized in Pielou (1977). References to methods and applications in the marine benthic environment will be found in Gage and Coghill (1977). The interaction of sample unit size and the scale of heterogeneity has been shown to materially affect the ability of a sampling program to measure aggregation and the degree to which aggregation influences estimates of community parameters (Lloyd, 1967; Morisita, 1962; Iwao, 1972; Grieg-Smith, 1964; Goodall, 1974).

Techniques used to evaluate the dispersion of marine organisms have included grids or lines of contiguous quadrats (Gage and Coghill, 1977; Heip, 1975; Angel and Angel, 1967; Jumars et al., 1977), paired cores randomly positioned in the intertidal (Gardefors and Orrhage, 1968), distribution mapping with closely spaced sample units (Kenchington and Hammond, 1978; Paine, 1963), spatial autocorrelation (Jumars et al., 1977) and cores with increasing separation (Thistle, 1978; Hogue, 1977). Small scale distribution mapping has been used in plankton studies (Wiebe, 1970; Cassie, 1959). Spectral analysis has been applied to plankton distributions in a few studies where many samples were available (Denman and Platt, 1974).

Diversity Measures

The community composition parameters which collectively can be referred to as diversity and evenness measures express three aspects of the composition of a community: the species richness, or number of species; the relative abundance of each (as evenness or dominance); and the absolute density of all organisms at the site. Most express either the first and second, or the second alone and a few express all three. Diversity is defined such that it increases with species richness, evenness of relative density, and overall density.

Diversity indices fall into several categories by their source. These may be termed the information group, derived from information theory; the Simpson group, based on probability theory; the McIntosh group, derived from a continuum concept of communities; and a miscellaneous group of varied origin. Within each of these groups, a set of indices can be defined which express the species richness and the evenness components of diversity, and the evenness component alone. A few cases also express the overall density of organisms. A summary of the indices and the component or components of diversity which each expresses is presented in Table IV. Not all indices are included, as the number of available indices proposed is large (eg. Smith and Grassle, 1977) and many are variants on other indices. Sources of information on the meaning of many diversity and evenness measures are Pielou, 1975 and 1977. Before planning sampling for the use of any index, the exact composition of the index, and the factors to which the index is responsive should be clearly understood. Indices, even those which express the same components of diversity, differ in their sensitivity to rare versus common species, and may differ in the degree to which evenness or species richness control the value of the index. The selection of a suitable index should be controlled by the emphasis which the individual worker wishes to place on rare species, and on the different aspects of diversity.

In general, diversity measures are applied to community studies for two reasons. The first is to compare sites to determine the extent to which sites are replicates, as discussed earlier. The second is to detect differences between control and sites affected by disturbances, or between pre- and post-disturbance stages at a single site. The assumption is that disturbance of a site will affect the species composition or the relative abundance of species in some manner detectable by changes in a diversity index. For example, if a pollution event results in dominance of a single resistant species, while most other species are severely reduced in number, the diversity of the site should be reduced relative to earlier measurements, or to control areas which are not in range of the pollution. However, it is also possible to have increases in diversity following pollution, as might occur when a dominant species is susceptible to pollution, and reduction of the dominant's density allows other species to increase in numbers. In such cases, the evenness and species richness might both increase. Diversity indices calculated on the basis of species richness and numerical density would not reflect changes in the reproductive capacity or health of organisms, until these changes influenced either species present or abundance of species.

A phenomenon which has been repeatedly observed (Dayton, 1975; Paine, 1974; Loya, 1976) is the successive increase and decrease in diversity following a disturbance. The apparent cause is removal of the dominant species, colonization of ephemeral and fugitive species, and subsequent reestablishment of the dominance of the major species. Pollution or other disturbances may be expected to cause a sucessional pattern in the dominant species which will be reflected in increases and decreases in diversity measures over long periods of time. If, however, the community which was disturbed was not in equilibrium to begin with, there may not have been dominant species, and changes in diversity would be unpredictable. A change in a diversity measure cannot be interpreted as indicating a disturbance effect of a particular event unless supporting evidence indicates that the diversity was stable prior to the event, or possibly was changing in some entirely different manner. Diversity may also change seasonally, as species are introduced and are unsuccessful, or as the abundance of species increases due to influx of young individuals. Seasonal patterns would have to be separated from changes due to disturbance.

TABLE IV

DIVERSITY INDICES

Name	Discussion	Symbol	Expressing: 1,2,3*
species richness		S	1 only
Margelef's index	Margelef (1957)	d	1 only
log-normal indices	Preston (1948)	$S^*=S_o$, a	1 and 2
Brillouin's index	Brillouin (1962)	B	1, 2, and 3
		H	1 and 2
Shannon's index	Shannon and Weaver (1963)	H'	1 and 2
Redundancy	Shannon and Weaver (1963)	R	2 only
Pielou's J (evenness)	Pielou (1975)	J	2 only
Lloyd's evenness	Lloyd, Zar, and Karr (1968)	π, r	2 only
Simpson's index	Simpson (1949)	λ, or D	1 and 2
" evenness	Simpson (1949)	E_s or J_s	2 only
" redundancy	Simpson (1949)	R_s	2 only
Hurlbert's index	Hurlbert (1971)	PIE	2 only
McIntosh's index	McIntosh (1967)	MDI	1, 2, and 3
" evenness	McIntosh (1967)	J_m	2 only
" redundancy	McIntosh (1967)	R_m	2 only

*1: Species richness

2: Evenness or dominance

3: Absolute density

Similarity measures

A large number of similarity measures have been proposed. Many are discussed in detail in the standard reference by Clifford and Stephenson (1975). These may be divided into a limited number of categories by their derivation. These are:

1. Similarity measures - ratios of shared attributed of two sites to the total number of attributes possible in the comparison.

2. Association measures - measures of the correlation of attributes of pairs of sites.

3. Euclidean distance - the distance separating two sites when the species are axes and their abundances coordinates in the S-dimensional space.

4. Diversity measures - compare the sites in such a way as to minimize the within-group diversity as sites are successively fused.

5. Probability measures - estimates of the probability that two sites are as similar as those under consideration, assuming that they came from the same superpopulation.

The attributes of similarity measures (using the term in the general sense rather than the restricted meaning favored by Clifford and Stephenson) vary greatly. Some emphasize dominant species, others rare species. Several can be used which require only species lists for two sites, assuming that the same effort of sampling was applied to both sites. This sort of data would be particularly easy to obtain by brief surveys, and therefore would be possible to obtain in preparation for selection of control and replicate sites. Use of simple similarity indices to select replicate sites would greatly increase the probability that disturbance events would be detected, since true replicate sites would have a greater probability of responding in the same manner to disturbance.

Similarity measures are also commonly used to cluster sites. In essence, clustering methods group sites according to the degree of similarity of sites, either by successively more similar sites being grouped, or by successively less similar sites being grouped. If some sites are disturbed and others are not, at some level of the clustering, the disturbed and undisturbed sites should fall into clearly separate clusters. Clifford and Stephenson (1975) discuss several clustering strategies. Examples of the use of similarity indices to cluster communities are given in Boesch (1977), Hummon (1974), Field and McFarlane (1968), Rosenberg (1976), Stephenson, et al (1971) and Littler and Murray (1975). The last is specifically an attempt to evaluate the effects of sewage outfall on rocky intertidal organisms, and is a good representative example of the type of program which must be conducted to fully evaluate the effects of disturbances on marine communities. Another good example of the application of similarity analysis methods to the detection of pollution effects in the marine environment is the evaluation by Rosenberg (1976) of the response of benthic marine communities to the closure of a sulfite pulp mill and the cessation of waste liquor discharge.

Advanced Data Analysis Methods

Several numerical methods are available but not commonly used for the assessment of disturbances, or the relation of species to environmental factors. Ordination is a method of ordering the data in such a manner as to permit identification of the major factors controlling distributions of species. An example of the use of ordination is given in Hughes and Thomas (1971 a, b). Clifford and Stephenson (1975) detail methods of ordination. Various methods for ordination of benthic communities are evaluated by Erman and Helm (1971).

Canonical analysis is a method of relating two sets of data, in order to identify aspects of the variability of both which appear to be related, and which might be caused by the same factor, or which may be used to infer that one set of variables caused some of the variation in the other set. Canonical analysis is discussed in Clifford and Stephenson (1975); and in Cooley and Lohnes (1971), who provide programs in FORTRAN IV for canonical analysis. An example of the use of canonical analysis is given in Read and Renshaw (1976), who compare beaches in different stages of pollution for the relationship of species to environmental variables such as salinity and tidal height.

More familiar is the use of analysis of variance to compare the abundances of species in different locations. The best example in the intertidal literature at present is the study by Myren and Pella (1977), examining the natural variability in the distribution of a bivalve at a beach subject to potential oil pollution. The ANOVA methods used in this paper are not extremely advanced, but are possibly beyond the immediate capabilities of the average worker. The degree of refinement in this study is such that the probability of detection of disturbances to the bivalve population is high.

A second method which is more familiar to the average worker is univariate regression. This, like canonical analysis, is useful in comparing two sets of data and finding correlations between variables. Any of a number of standard statistical textbooks, such as Snedecor and Cochran (1967), are suitable sources for details of linear regression methods. An excellent text is that of Neter and Wasserman (1974), who discuss regression methods in great detail. An example of the use of regression methods is given in Phillips (1976), who examined the effects of metallic pollutants on mussel tissue weights as an indicator of pollution effects.

Multivariate regression methods may be used instead of univariate regression methods when the response of entire sets of variables to independent variables is to be examined. The text by Cooley and Lohnes (1971) gives both a theoretical description of multivariate analysis, and examples and programs for application of the methods.

Allen (1971) examined the use of multivariate analysis in studies of terrestrial algae. Moore (1973, 1974) used multivariate methods to examine the kelp holdfast fauna of northeast Britain. Cassie (1972) provides a computer program for multivariate statistical analysis. Additional information on complex computer programming in ecology is given in Davies (1971). An excellent guide to the detailed application of these methods in pollution effect studies is the manual by Boesch (1977) in this report series.

Recruitment and Productivity

Recruitment

The relative abundance and number of species, summarized in diversity statistics, have been the most commonly used community parameters in the assessment of the effects of pollution on marine organisms (e.g. Littler and Murray, 1975). The assumption underlying the use of diversity measures in assessing pollution effects is that the number of species or the relative abundance of species will be affected by pollution within the period over which the area is studied. In many cases, mature individuals are much more resistant to pollutants than larval or juvenile individuals. Studies which ignore the effects of pollution on the rate of recruitment may underestimate the magnitude of the damage.

There are two major ways in which the recruitment rate may be affected. The reproductive capacity of adults may be reduced by a decrease in the overall health of mature individuals or by some more specific effect. The success rate of newly recruited individuals may also be decreased if the larval or juvenile stages are more sensitive to pollutants than are the adults. Either of these can be important. A decrease in the successful recruitment of new individuals to the population may not be detectable as a change in the population density for some time afterward. The effects will be greatest when the adults have a short lifespan and would normally be replaced by a new generation on a yearly or seasonal basis. Long-lived species are more likely to recover from pollution damage and reproduce normally. Obviously, the magnitude of the effects of a pollution event will depend on the coincidence of the event and the reproductive cycle. Toxins during the period of larval dispersal may kill most of the larvae, while pollution in the non-reproductive season may allow surviving adults to recover and reproduce normally. It is useful to know in advance whether the dominant species reproduce yearly, and whether their lifespan and susceptability to toxins make them vulnerable to pollution at critical periods of the year. Baseline studies should attempt to evaluate the recruitment patterns of the major species.

If size data are calculated, the recruitment of small and presumably young individuals can be determined. Many species reproduce seasonally and show a typical pattern of a seasonal peak in the smallest size classes. Comparison to control areas or to previous data in the same areas should indicate whether a given frequency of small individuals represents the normal recruitment to the population. If the peak is followed through time, the decrease in numbers of individuals remaining from the initial recruitment can be compared to the normal mortality rate, to evaluate whether young individuals are suffering an abnormally high mortality. If recruitment is not strongly seasonal, there may be a constant input of new individuals at some typical rate, and the observed frequency of small individuals can be compared with the normal frequency at control locations.

It is essential that evaluation of the recruitment rate recognize that there are normal variations in the reproductive success of species. Some species show patterns of relatively infrequent successful "sets", such as mussels (Paine, 1974). The normal variation of recruitment must be separated from the variation due to catastrophic events. If the recruitment to a control area remains within the limits of normal variation, a reduction in the recruitment to a study area is probably due to the event, but may also indicate that the control area is not suitable (other causes may be producing the reduction). If the recruitment to a control area is reduced by an amount similar to the study area, then the reduction is probably not due to the pollution event at the study area. If the history of variation at the study area is available, a reduction in recruitment should not be considered unless it is greater than the expected year-to-year variation. The significance of the reduction can be evaluated by the usual statistical methods, given estimates of the variability in recruitment through time. If changes in recruitment success were within normal limits, it would be necessary to prove that a control area did not show the same reduction in order to prove that the reduction, though not abnormally large, was due to some pollution event. This points out the value of having both a monitoring history of a location, and control areas. The difficulty of interpreting short-term impact studies because of long-term variability is well demonstrated by Boesch et al. (1976) who review the dynamics of estuarine benthic communities. The macrobenthic fauna of estuaries show substantial dynamic seasonal and long-term variability caused both by life history features and long-term habitat changes.

Productivity

It is important to separate estimation of the standing stock from estimation of the production of an area (Allen, 1971). The standing stock is the biomass present at a given time. Single samples in time measure the standing stock at that time. The production of an area is the amount of organic material produced through growth and reproduction of individuals. It is possible to have a high production rate and a low biomass, if the production is consumed rapidly. The major effect of pollution could be to decrease the production rate rather than the biomass, so that the effects on biomass would not be immediately evident. Populations also go through normal fluctuations in biomass as a result of seasonality of production. Deviations from the normal pattern of changes in the production and the biomass present may be good indicators of pollution effects. Bellamy et al. (1972) discuss the utility of productivity data in pollution monitoring studies. The following are some examples of ways in which pollution effects on production may be detected.

Many species have highly seasonal recruitment and show strong fluctuations in biomass and mean size of individuals. The fluctuations will be most pronounced when the adults die after reproducing, such as occurs in some amphipod species on west coast sandy beaches (Bosworth, 1976). A large part of the production of these populations goes into reproduction during spring. In the remainder of the year most of production goes to growth. Pollution effects could be detected through decreases in the population in the following year due to failure of the larval recruitment (larval deaths) or to diminished reproductive capacity of adults (adult health), or perhaps due to a decrease in the number of adults reaching reproductive size. These effects would be in addition to the death of adults immediately following pollution events. Pollution effects on growth could be examined through following the newly recruited cohort of juveniles as they increase in size.

When the adults do not die after reproducing, a bimodal distribution in size often results. The larger mode consists of older adults. The smaller size mode represents newly recruited juveniles. The juveniles could be identified for growth rate estimates until their mode merged with the adult size mode.

Species which have continual reproduction often have a stable distribution of age or sizes. The frequency of individuals in any given age or size class is roughly constant, provided that conditions are stable. A pollution event might be expected to alter the stable age distribution, if recruitment is affected. Deviations from the typical distribution of sizes would be indicative of disturbances to the population.

The second major component of production is growth. The growth of individuals can be decreased without death, reducing the biomass present without changing the numerical abundance. It has already been implied that in some cases, a reduction in the growth rate could reduce reproductive capacity of the population by decreasing the number of individuals which reach reproductive size. Ideally, growth rates can be measured for many species either directly (see next section), or indirectly through following the average size of a year class of newly recruited juveniles.

Health of individuals may also be affected. In most cases these effects will be difficult to measure quantitatively in field sampling and data may be more in the nature of qualitative observations on the appearance of collected specimens. In some cases, the quantity of certain storage products such as lipids may be useful measures of the health of individuals. Another measure is the weight of reproductive tissue. Unusual behavior may often result from poor health, but would seldom be quantifiable. The regression of weight per individual on some independent measure of the linear size of individuals may indicate if a species is strongly affected by pollutants, in that one might expect that individuals of a given size may be of lesser weight than usual.

Measurement of Production

Methods for the measurement of production may be divided into long and short term techniques. Methods which measure the production of either animals or plants over long periods generally measure the change in weight of the individuals tested or of populations on the average. Short-term estimates usually test the rate of uptake of some substance over a period of hours.

Bittaker and Iverson (1976) compared the effectiveness and interpretation of two relatively short-term methods, applied to the seagrass Thalassia testudinum. The methods compared were the Wetzel (1964, 1965) adaptation of the Steeman-Nielsen (1952) C^{14}-uptake technique, and the Zieman (1974, 1975) method for staple-marking of growing plants. The former method measures the in situ uptake of radioactive carbon over a period of several hours, giving an estimate of the rate of carbon particulate production over that period. The latter method measures the rate of lengthening of blades over a period of four to six days, and estimates the dry weight production of leaves (blades). Details of the methods and numerous cautions on the application of the methods are given in Bittaker and Iverson (1976) as well as the original sources. This paper also provides an excellent bibliography for productivity measurement for seagrasses and for benthic macroalgae. Many of the methods available for the measurement of macrophytic production are described by Round and Hickman (1971), who consider the estimation of production from increases in area, volume, wet weight, dry weight, ash-free dry weight, and length (as measured by stapling or by punching holes). The problems of the various methods are discussed and C^{14} methods are also described. Similar papers giving some methods for macroalgal production are Westlake (1969) and Ott and Maurer (1977). Discussions of productivity measurement for algae in special circumstances, such as in sands or overlying mud, may also be found in Sournia (1976, sands), Steele and Baird (1968, sands), and Leach (1970, mudflats). The seasonality of rocky intertidal macroalgal standing crop, density, growth and reproduction, and methods for their measurement are discussed in Hansen and Doyle (1976) and Mathieson et al. (1976).

Over long periods, the simplest method for the estimation of production involves estimation of the rate of intake of energy, and the sum of the rates of energy expenditure. Given a feeding rate, the production of a population may be summarized as the sum of growth, reproduction, and mortality. The difference between the gross intake and the net production goes to maintenance or is lost as excretion or egestion. An example of the construction of an energy budget is given in Kay and Brafield (1973) for the polychaete Neanthes virens. Other examples of the estimation of littoral macrofaunal production are Hibbert (1976), Burke and Mann (1974), Warwick and Price (1975) and Hughes (1970, a and b) on bivalve and gastropod production; and Paine (1971) on production of a gastropod. Numerous other studies have examined aspects of the total flow of energy in populations without construction of estimates of energy flow and production rates. A total energy budget is necessary to estimate the true production of a species. Crude estimates of the production of populations can be derived from the increase in biomass of the population over a period of time, without consideration of the rates of mortality and reproduction. These estimates underestimate the amount of biomass produced by the population by ignoring the biomass which was utilized by predators or scavengers, as well as production in the form of gametes, which is also largely returned as food for the rest of the community. In many cases, the time and economic limitations of a program will prevent complete definition of the energy flow in the dominant species, let alone the entire community. These studies should attempt to estimate the production of the major species as well as the available information allows. Most programs should be able to document the change in standing stock of major species, and estimate the recruitment to these species. The mortality rate may not be available from the standard sampling program. Simple estimates of mortality rates may be obtainable from the rates of loss of marked individuals from defined permanent quadrats, or from the difference between the potential number of adults resulting from one season's recruitment and the actual number of individuals which survive to adulthood. Ebert (1972) gives methods for the estimation of growth and mortality from size data. The studies of Nichols (1975, 1977) illustrate how dynamic aspects of production can be measured from field sample data alone. Crisp (1975) and Mann (1969) provide good short reviews of secondary productivity and conversion factors, as well as production/biomass ratios for some littoral benthic invertebrates.

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